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Conservación del nicho ecológico en topillo de Cabrera (Microtus cabrerae). Aproximaciones ecológicas y filogenéticas

MEMORIA PARA OPTAR AL GRADO DE DOCTORA

PRESENTADA POR

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Conservación del nicho ecológico en topillo de Cabrera (*Microtus cabrerae*). Aproximaciones ecológicas y filogenéticas

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Índice

Abreviaturas

AIC	Akaike information criteria
AUC	Area-under-the-curve
BAM	Biotic, abiotic and movement factors
BM	Brownian motion
BMR	Basal metabolic rate
CCSM	Community climate system model
CLM	Community-level models
Cm	Minimal thermal conductance
DNA	Deoxyribonucleic acid
GCM	Global circulation model
GLM	Generalized linear model
ka BP	*Kilo annum*, Thousand years before the Present
LGM	Last Glacial Maximum
LIP	Last Interglacial Period
LMM	Linear mixed model
M_b	Body mass
Maxent	Maximum entropy
MIS	Marine isotope stage
MR	Metabolic rate
NDVI	Normalized difference vegetation index
NPP	Net primary productivity
Po_2	Partial pressure of oxygen
PCA	Principal components analysis
PNC	Phylogenetic niche conservatism
PND	Phylogenetic niche divergence
RF	Random forest model
RMR	Resting metabolic rate
SDM	Species distribution model
Ta	Environmental temperature
Tb	Body temperature
Tlc	Lower critical temperature of thermoneutrality
TNZ	Thermonuetrality zone
Vo_2	Rate of O_2 consumption
ΔTm	Minimal (body-ambient) termal differential

Resumen

Introducción

En la actualidad, el cambio climático global ya es una realidad y sus efectos son patentes sobre la biodiversidad. Este nuevo escenario impone unas condiciones ante las que las especies deben adaptarse o migrar, como medio de supervivencia, o, en caso de que el cambio climático supere sus capacidades, podrían extinguirse. Bajo este marco, se incrementa el interés por esclarecer la relación entre el ambiente y la distribución de las especies, de ahí que exista un auge de los estudios de nicho ecológico. Gran parte de las aproximaciones que han abordado estos estudios se han fundamentado en la asunción de la conservación del nicho, es decir, la tendencia de los taxones a retener las condiciones de los nichos ancestrales.

No obstante, la literatura ha presentado evidencias mixtas que no permiten establecer un patrón a favor de la conservación o la evolución del nicho, lo que podría ser un hándicap en las asunciones de muchos modelos predictivos. Por tanto, el estudio de la conservación del nicho requiere un enfoque amplio en el que se integren tanto trabajos a escalas temporales reducidas que focalicen sobre rasgos medibles en los organismos; como investigaciones longitudinales en las que se analizan rasgos asociados a las especies (a lo largo de filogenias) y los factores abióticos en amplios periodos de tiempo. A través de cada uno de ellos se profundiza en las diferentes dimensiones del nicho.

Este estudio de la conservación del nicho se encuentra acotado sobre el taxón *Microtus cabrerae* (Rodentia, Cricetidae), una especie especialista del hábitat y endémica de la península Ibérica que presenta una distribución en contracción. Se incluye en la subfamilia Arvicolinae, un grupo con un relativamente corto periodo de divergencia y, a la par, una alta tasa de especiación. Estos factores convierten a los arvicolinos y, en particular, a *M. cabrerae* en interesantes modelos para evaluar la conservación del nicho.

Objetivos

Esta Tesis gira en torno al objetivo principal de la identificación de patrones que indiquen la conservación o evolución del nicho en *M. cabrerae*. Para ello, se integra un amplio espectro de

dimensiones del nicho con el fin de conseguir evidencias complementarias que sirvan para esclarecer la tendencia conservadora o divergente en el nicho de la especie.

Metodología

En primer lugar, se desarrolló un experimento ecofisiológico que no contribuyó directamente a la dilucidación de la conservación o divergencia del nicho, pero resultó imprescindible para los siguientes trabajos desarrollados en esta Tesis. Para ello se empleó un instrumento de medida metabólica (respirómetro) que permite registrar la tasa metabólica de la especie frente a la variación del ambiente térmico, a fin de establecer la tasa metabólica basal y la región de termoneutralidad.

En el segundo experimento de ecofisiología se desarrolló un estudio de jardín común. Se capturan 19 individuos de *M. cabrerae* de diferentes poblaciones, distribuidas en dos pisos bioclimáticos y se les sometió a la medida de la tasa metabólica basal. A continuación, los animales fueron mantenidos en las condiciones de jardín común con condiciones ambientales idénticas. Finalmente, se repitió la medida de la tasa metabólica basal. Además, se planteó un experimento de cría en cautividad con el fin de medir la tasa metabólica basal sobre animales de la primera generación filial nacidos bajo condiciones controladas.

Con el tercer trabajo se desarrolló un estudio longitudinal que hace uso del registro fósil de *M. cabrerae* a lo largo de su historia. Se seleccionaron los tres principales cambios climáticos acontecidos en la historia de la especie: el Último Interglacial, el Último Máximo Glacial y el Holoceno Medio, sobre los que se obtuvo el registro fósil de *M. cabrerae*. Cada uno de estos escenarios se comparó con la distribución actual de la especie. Para esta investigación se aplicó un doble enfoque metodológico de transferencia de modelos y medida de solapamiento de nichos. La transferencia temporal de modelos se ejecutó en dos sentidos: calibrando en la actualidad para transferir al pasado (se evaluó con el registro fósil) y calibrando en los paleo-escenarios para transferir al presente (se evaluó con la distribución actual). La evaluación del espacio ambiental se basó en las comparaciones entre el nicho actual de la especie y los paleo-nichos para obtener medidas de solapamiento, los test de equivalencia y similitud y las dinámicas de los nichos.

El último trabajo integró la información ambiental de los nichos con la filogenia de los arvicolinos. A partir de esta filogenia se establecieron un total de 23 pares de especies hermanas

entre las que se comparó el solapamiento de sus nichos, los test de equivalencia y similitud y las dinámicas de los nichos. Complementariamente, se evaluó la correlación entre distancias ambientales y filogenéticas por medio del test de Mantel.

Resultados

En base a las experiencias ecofisiológicas se extrajo que las tasas metabólicas, además de tener un importante componente de base filogenética, se encontraban acopladas a las condiciones ambientales. En este sentido, las condiciones de aridez impusieron una limitación sobre las tasas metabólicas basales y llevaron a la especie a iniciar la zona termoneutra a mayores temperaturas e incrementar la conductividad térmica para disipar el exceso de calor. Además, la experiencia del jardín común permitió identificar que el mecanismo subyacente en el acople fisiológico entre las poblaciones y los ambientes de los dos pisos bioclimático no era lábil, sino que podría encontrarse fijado genéticamente. Sin embargo, el fracaso en la experiencia de cría en cautividad, no nos permitió descartar otros mecanismos de ajuste fisiológico no genéticos irreversibles.

A través de la aproximación basada en el espacio ambiental se pudo esclarecer la respuesta de *M. cabrerae* ante la sucesión de cambios climáticos. En ninguno de los casos los nichos fueron equivalentes y, sólo de forma parcial durante el Último Máximo Glacial y totalmente en el Último Interglacial, los paleo-nichos fueron más similares al nicho actual que por azar. El calentamiento climático impuesto durante el Último Interglacial dejó restringida a la especie a un espacio ambiental más limitado que el que actualmente ocupa.

La comparación del espacio ambiental a lo largo de la filogenia de los arvicolinos reflejó una tendencia general de bajo solapamiento que indica que los nichos de las especies se habrían alejado de las condiciones del nicho ancestral. Sólo cuatro pares de especies hermanas mostraron un solapamiento significativo, indicativo de que en ellas se ha producido una selección de espacios ambientales similares a las condiciones del nicho ancestral.

Conclusiones

La existencia de un acople fisiológico intraespecífico a las condiciones abióticas de los pisos bioclimáticos apunta a un probable mecanismo subyacente de adaptación genética local,

aunque no se pueden descartar otros ajustes fisiológicos no genéticos e irreversibles. Siguiendo este patrón, la evaluación del nicho de *M. cabrerae* a lo largo de su historia revela periodos en los que la especie ocupó espacios ambientales diferentes a los actualmente tolerados. Por tanto, la especie habría sufrido cambios en su nicho que podrían ser explicados por procesos adaptativos a las condiciones ambientales. No obstante, ante un escenario de calentamiento el nicho de la especie quedó restringido con respecto al actual lo que indica que estas condiciones severas se encontrarían en el límite de tolerancia de *M. cabrerae*. En la misma línea, la evaluación de la filogenia de los arvicolinos apoya el alejamiento del nicho de la especie de las condiciones del nicho ancestral y, de ahí, la evolución del nicho.

En consecuencia, el análisis conjunto de los experimentos ecofisiológicos y la evaluación de los espacios ambientales a lo largo de la historia evolutiva de la especie y su filogenia, nos aporta una evidencia acumulada con la que descartar la conservación del nicho en *M. cabrerae*. Por todo ello, la asunción de la conservación de nicho no puede ser una cualidad inherente a las especies.

Summary

Introduction

Currently, the global climate change is not only evident, but its implications on biodiversity are already patent. This new scenario imposes conditions in which species must adapt or migrate, as a means of survival, or, when climate change exceeds their abilities, they could be extinguished. Under this framework, the interest to clarify the relationship between the environment and the distribution of the species increases, hence there is a rise in ecological niche studies. Many of the approaches used in these studies have assumed of niche conservation, that is, the tendency of taxa to retain the conditions of ancestral niches.

Nevertheless, the literature has presented mixed evidences that do not allow establishing a pattern in favour of the niche conservatism or shift, which could be a handicap in the assumptions of many predictive models. Therefore, the niche conservatism study requires a wide approach integrating both short-term focused on measurement traits of organisms; and longitudinal investigations in which species' traits are analyzed (throughout phylogeny), such as the abiotic factors, throughout long periods of time. Each of them delves into the different niche dimensions.

This study of niche conservatism is focused on *Microtus cabrerae* (Rodentia, Cricetidae), a habitat specialist species and endemic from the Iberian Peninsula with a contraction in its distribution. It is included in the Arvicolinae subfamily, which has experienced a high speciation rate in a relative short time of divergence. These factors make of the arvicoline and, especially, the *M. cabrerae* in interesting models to evaluate the niche conservatism.

Objectives

This Thesis revolves around the main objective of the identification of indicative of the niche conservatism or shift in *M. cabrerae*. Hence, it is integrated a wide spectrum of the niche's dimensions in order to obtain complementary evidences with which clarifying the conservative or divergent trend in the niche of the species.

Methods

Firstly, it was developed an ecophysiological experiment which did not directly contribute to the elucidation of niche conservatism or divergence, but it was essential to provide the data for the following works of this Thesis. The metabolic response against the thermal environmental variation was record with a metabolic instrumental (the respirometry), with the aim of determining the basal metabolic rate and the thermoneutral zone.

In the second ecophysiological experiment a common garden study was developed. A total of 19 individuals of *M. cabrerae* from different subpopulations were captured, from animals distributed between two bioclimatic zones, and they were measured in the respirometry to determinate their basal metabolic rate. Following, the animals were subjected to common garden conditions with identical environmental conditions. Finally, the measurement of basal metabolic rate was repeated under the common garden conditions. Besides, a breeding experiment under common garden situation was projected with the aim to measure the basal metabolic rate from animals of the first filial generation born under control conditions.

The third chapter was based on a longitudinal study which used the fossil record of *M. cabrerae* throughout its history. The three main climate changes occurred in the history of the species were selected: The Last Interglacial, the Last Maximum Glacial and the Mid-Holocene, on which the fossil record of *M. cabrerae* was obtained. Each one of these scenarios was compared to the current distribution of the species. In this investigation it was applied a double methodological approach of model transferability and the measurement of niche overlap. The temporal transferability of models was executed in two senses: calibrating the model in the current scenario to transfer it to the past (it was evaluated with the fossil record) and calibrating them in the paleo-scenarios to transfer them to the present (they were evaluated with the current distribution). The evaluation of the environmental space was based on the comparisons between the current niche and the paleo-niches to get the overlap measurements, the equivalency and similarity tests and the niche dynamics.

The last study integrated the environmental information of the niches with the Arvicoline phylogeny. The comparison of niche overlap, equivalency and similarity tests and the niche dynamics were evaluated in a global of 23 sister species of the Arvicoline phylogeny. Complementarily, the correlation between the environmental and phylogenetic distances was assessed with the Mantel test.

Results

The ecophysiological experiments provided two important results: the metabolic rates and their coupling to the environmental conditions, although they also had an important phylogenetic base. In this vein, the aridity limited the basal metabolic rates and provoked the delay of the beginning of the thermoneutral zone and the increase in thermal conductivity to dissipate the excess heat. In addition, the common garden allowed to identify that the underlying mechanism in the physiological coupling between the subpopulations and the environmental conditions of the bioclimatic zones was not labile, it could be fixed genetically. However, the failure in the captive breeding experiment did not allow us to rule out other mechanisms of physiological adjustment of irreversible non-genetic type.

The approximation based on the environmental space clarified the response of *M. cabrerae* to the climate changes. In none of the scenarios, the niches were equivalents and, only in part during the Last Maximum Glacial and totally in the Last Interglacial, the paleo-niches were more similar to the current niche than would be expected by chance. The warming climate of the Last Interglacial limited to the species to a more restricted environmental space than the one currently occupied.

The comparison of the environmental space throughout the Arvicoline phylogeny revealed a general trend of low overlap, which indicated that the species' niches would have distanced from the ancestral niche. Only four sister species pairs had a significant overlap, indicating a selection of environmental spaces similar to the ancestral niche conditions.

Conclusions

The intraspecific physiological coupling to the abiotic conditions of the bioclimatic zones points to a possible underlying mechanism of local genetic adaption, although other irreversible non-genetic physiological adjustments cannot be discarded. In line with this pattern, the niche assessment of *M. cabrerae* throughout its history reveals periods in which the species occupied different environmental spaces to the currently tolerated. Therefore, the species would have undergone niche shifts that could be explained by adaptive processes to the environmental conditions. Notwithstanding, under a climate warming scenario the species' niche was more restrictive than the current one, which indicates that these severe conditions would reach its

tolerance limit. In this vein, the Arvicoline phylogeny evaluation supports the distance of the species' niche from the ancestral niche conditions and, hence, the niche evolution.

Consequently, the global analysis of the ecophysiological experiments and the environmental space assessment throughout the species' evolutionary history and phylogeny provide us accumulated evidence with which to discard the niche conservatism in *M. cabrerae*. For all these reasons, the niche conservatism assumption cannot be an inherent quality of the species.

Introducción general

A principios de la década de los 70s la comunidad científica puso el foco sobre la existencia de un cambio climático forzado por la actividad antrópica (Landsber, 1970; Kopec, 1971; Cooper, 1978), especialmente, a causa del incremento en las concentraciones de gases de efecto invernadero (IPCC, 2007). En la actualidad, el cambio climático global ya es una realidad y sus efectos son patentes sobre la biodiversidad, como: cambios en los límites de los rangos de distribución, abundancias de especies o cambios fenológicos (Parmesan & Yohe, 2003; Root *et al*., 2003; Parmesan, 2006).

Los patrones de biodiversidad y la distribución de las especies se encuentran en equilibrio con el clima (Root, 1988), de ahí que, las alteraciones climáticas conlleven impactos sobre ambos. La supervivencia de las especies depende de su capacidad para afrontar las nuevas condiciones, para ello algunas especies presentan una alta capacidad de adaptación. La adaptación local y la plasticidad fenotípica son los principales mecanismos intrínsecos de las especies con los que poder hacer frente al estrés ambiental (Benito Garzón *et al*., 2011). Alternativamente, a través de la migración las especies con limitada capacidad adaptativa pueden rastrear el territorio en busca de las condiciones climáticas pérdidas. En concreto, se han estimado cambios promedio en los rangos distribucionales hacia latitudes más septentrionales de 6.1 (± 2.4) km/década o de 1 m/década en ascenso altitudinal (Parmesan & Yohe, 2003). Sin embargo, la peculiaridad de este cambio climático global reside en la rapidez con la que se ha desencadenado todo el mecanismo de cambio climático (Jouzel *et al*., 2007). Por ello se estima que grandes áreas del planeta requerirán velocidades de migración superiores a las estimas más optimistas, a fin de que las especies puedan mantener las condiciones de sus nichos (Loarie *et al*., 2009). Como consecuencia, sobre especies con baja capacidad adaptativa o insuficientes tasas de migración, el cambio climático podría desencadenar procesos de extinción (Thomas *et al*., 2004). Un ejemplo lo representa el mamut lanudo (*Mammuthus primigenius*), en el que el cambio drástico del clima entre el Final del Pleistoceno y el Holoceno provocó una reducción de su área de distribución del 90%, dejando a la especie en un estado de alta vulnerabilidad frente a un escenario de incremento de presión antrópica (Nogués-Bravo *et al*., 2008).

La relación entre el ambiente y distribución de las especies es una cuestión de notorio y tradicional interés en la Ecología (Krebs, 1972). En este contexto se acuña el concepto del nicho,

en el que se distinguen dos enfoques: el nicho *Grinnelliano* y el nicho *Eltoniano*. El primero atañe fundamentalmente a variables escenopoéticas, es decir, los factores abióticos (Grinnell, 1917; Soberón, 2007); en cambio en el nicho según Elton son más relevantes las variables bióticas que implican interacciones y dinámicas entre las distintas especies (Elton, 1927; Soberón, 2007). Hutchinson (1957) aunó ambos enfoques en un nicho entendido como el hipervolumen n-dimensional conformado por variables abióticas y bióticas donde la especie puede mantener un crecimiento demográfico positivo (Figura 1). La parte del nicho que comprende las variables abióticas o puramente físicas del medio circunscribe el nicho fundamental. A través de este nicho fundamental se relaciona la respuesta fisiológica de la especie ante las condiciones y las restricciones impuestas por del entorno (Guisan & Zimmermann, 2000), por tanto su aproximación debe partir de modelos mecanicistas (ej.: Kearney & Porter, 2009). Estos modelos desarrollan experimentos fisiológicos y comportamentales en campo o en laboratorio (Guisan & Thuiller, 2005) a fin de esclarecer los límites fisiológicos. La reducción sobre el nicho a causa de las interacciones bióticas y/o limitaciones a la dispersión de las especies da lugar al nicho realizado (Guisan & Thuiller, 2005). Para la estima del nicho realizado se debe recurrir al área de distribución de la especie, empleando sus localizaciones en campo (Guisan *et al*., 2017). Los modelos de distribución de especies (SDM, por sus siglas en inglés) son buenas herramientas para establecer el nicho realizado a partir de datos de presencia (Stigall, 2014). No obstante, cualquiera de las aproximaciones en las que se emplee al nicho de las especies debe ser considerado como una abstracción de la realidad que difícilmente permitirá describir de forma completa las condiciones que modelan los requerimientos ecológicos de las especies (Araújo & Guisan, 2006).

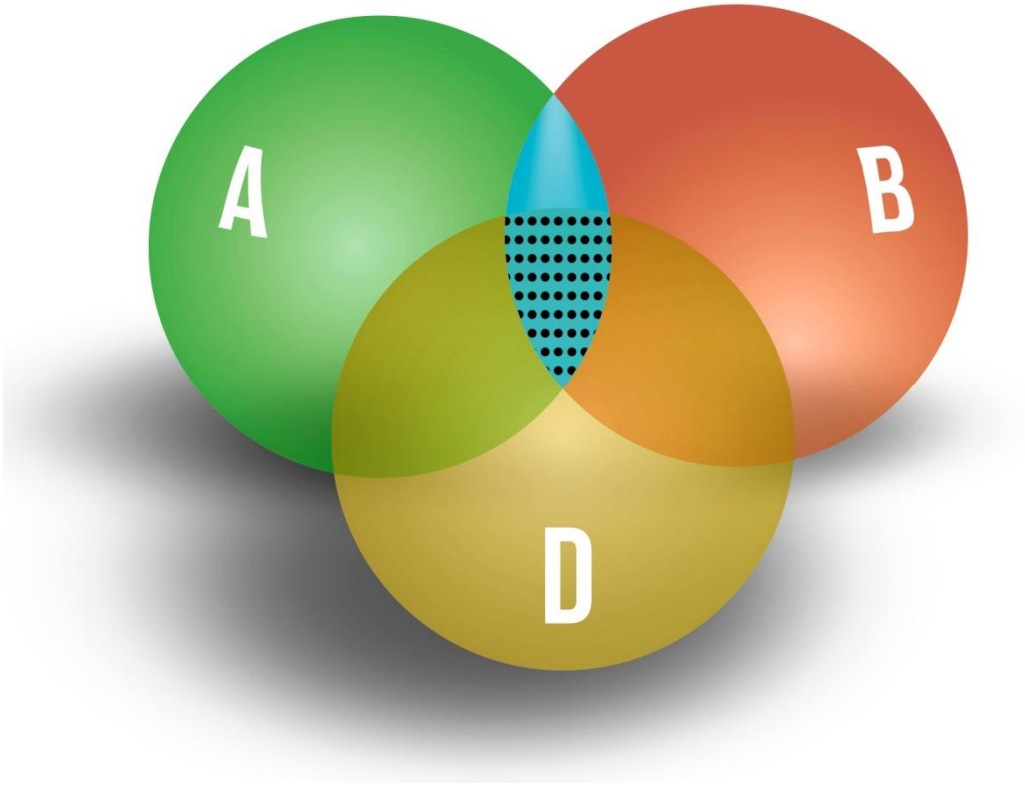

Figura 1. Diagrama con los n-hipervolúmenes que constituyen el nicho fundamental (área A: factores abióticos) y nicho realizado de la especie. La conjunción del área A (factores abióticos) y B (factores bióticos) delimita la distribución potencial de la especie (en azul), que queda restringida por el área D (dispersión), dando lugar al nicho realizado de la especie (espacio con trama).

En línea con el concepto de nicho, se ha desarrollado un extenso campo de investigación sobre las dinámicas del nicho ecológico que tratan de dilucidar el predominio de los mecanismos de conservación o evolución del nicho. A través de la idea de la conservación de nicho se establece una visión amplia sobre las relaciones entre ambiente y nicho en la que los taxones retendrían los rasgos del nicho ecológico a lo largo del tiempo y del espacio (Wiens *et al*., 2010). De modo más concreto, la conservación del nicho filogenético (PNC, por sus siglas en inglés) plantea la tendencia de que especies relacionadas filogenéticamente retengan los rasgos del nicho ancestral, por lo que sus nichos son ecológicamente más similares a lo largo del tiempo y del espacio (Wiens & Graham, 2005; Losos, 2008; Wiens *et al*., 2010; Stigall, 2014). En el extremo opuesto del espectro de la estabilidad del nicho se sitúa el cambio, evolución o divergencia del nicho filogenético (PND, por sus siglas en inglés), según el cual, especies emparentadas filogenéticamente no tienen mayor similitud en los rasgos del nicho, sino que en ellas se ha producido un proceso de alteración de sus rasgos ecológicos para crear nuevas oportunidades

(Stigall, 2014; García-Navas & Rodríguez-Rey, 2018). El auge del estudio de la conservación del nicho parte de la necesidad de esclarecer las capacidades de adaptación de las especies ante un escenario de rápido cambio climático y, a la vez, se encuentra favorecido por la creciente disponibilidad de bases de datos climáticas a escala global y la accesibilidad a herramientas informáticas para su análisis (Pyron *et al*., 2015; García-Navas & Rodríguez-Rey, 2018). En este marco, los modelos de distribución de especies son esenciales como herramientas predictivas de la respuesta de las especies a cambios ambientales. Sin embargo los SDMs están basados en la asunción de la conservación del nicho, por lo que comprobar si ésta prevalece tiene una enorme transcendencia aplicada más allá de la meramente académica.

La conservación de nicho se ha identificado sobre una amplia variedad de taxones: mamíferos, aves y mariposas mexicanas (Peterson *et al*., 1999), angiospermas (Prinzing *et al*., 2001), reptiles (Pyron & Burbrink, 2009), murciélagos tropicales (Buckley *et al*., 2010), especies de *Pedicularis* árticas (Tkach *et al*., 2014) o aves y mamíferos tropicales (Khaliq *et al*., 2015), entre otros. Igualmente son muchos los estudios que han revelado evolución del nicho sobre una alta diversidad de grupos taxonómicos (ej.: herbáceas (Broennimann *et al*., 2007), peces (Lauzeral *et al*., 2011), aves (Lawson & Weir, 2014; Pearman *et al*., 2014) o mamíferos (Blair *et al*., 2013; Khaliq *et al*., 2015)). Por tanto ante la existencia de evidencias mixtas, no se puede establecer un patrón a favor de la conservación o evolución del nicho (Münkemüller *et al*., 2015). En este sentido, la conservación o divergencia de nicho resulta una cuestión contexto-dependiente en función del taxón (Pearman *et al*., 2008; Cooper *et al*., 2011), la profundidad de estudio dentro de la filogenia (Stigall, 2014; Peixoto *et al*., 2017), el conjunto de variables empleado en la definición del nicho (Warren *et al*., 2008; Rödder & Lötters, 2009) o la escala temporal o espacial con la que se analice el nicho (Warren *et al*., 2008; Nogues-Bravo, 2009; Peterson, 2011). Además, el método empleado en el análisis de la conservación o divergencia del nicho podría también interferir en las conclusiones finales (Cooper *et al*., 2011). La diversidad de aproximaciones metodológicas incluye el empleo del registro fósil para comparar distribuciones actuales con paleo-distribuciones (ej.: McGuire & Davis, 2013), test filogenéticos para comparar la evolución de rasgos frente a lo que se esperaría con modelos evolutivos (ej.: García-Navas & Rodríguez-Rey, 2018), evaluaciones del espacio ambiental entre especies emparentadas mediante test de similitud y equivalencia (ej.: Aguirre-Gutiérrez *et al*., 2015) o test para relacionar distancias filogenéticas y distancias ambientales (ej.: Losos *et al*., 2003), entre otras.

El concepto del nicho es suficiente amplio como para que en el estudio sobre la conservación o evolución del nicho puedan considerarse la totalidad de rasgos funcionales de las especies

(Wiens *et al*., 2010). Los rasgos son una aproximación al desempeño del organismo, de ahí que, normalmente se encuentran definidos a nivel individual (Violle *et al*., 2007), sin embargo, en los estudios de nicho resulta habitual extrapolar este concepto a la especie o incluso a factores externos como las variables abióticas (ej.: Lv *et al*., 2016; García-Navas *et al*., 2018). En concreto, en esta Tesis se contemplan los rasgos como cualquier característica propia de una especie susceptible a alteraciones a lo largo del tiempo a consecuencia de control genético o asociado a cambios en otros rasgos (Cooper *et al*., 2010) y que permitan definir el espacio del nicho de la especie. La elección de unos factores en detrimento de los otros reside en el objetivo del estudio y la posibilidad de acceder a los rasgos a medir. Así, en trabajos que buscan patrones en escalas temporales reducidas es frecuente el empleo de rasgos medibles directamente sobre los organismos a través de experimentos de jardín común (o *common garden*) (ej.: Broggi *et al*., 2005; Jensen *et al*., 2008) o de transplante (ej.: Bartheld *et al*., 2015). En ellos, la incorporación de los rasgos fisiológicos resulta de gran relevancia en el estudio del cambio climático pues permite relacionar cómo varían los límites en los nichos fundamentales de las especies con las alteraciones del clima, establecer los posibles mecanismos de los organismos para afrontar esos cambios y comprender sus capacidades de respuestas evolutivas (Bozinovic *et al*., 2013). Sin embargo, no siempre es posible recurrir a este tipo de rasgos, especialmente en trabajos comparativos con otros grupos taxonómicos, puesto que requieren parámetros medidos bajo los mismos estrictos protocolos experimentales (Genoud *et al*., 2018). Otra de las limitaciones en el empleo de estos rasgos está en la escasez de rasgos fisiológicos contemplados en las bases de datos. Resulta habitual que se incluyan medidas como la tasa metabólica basal (BMR, por sus siglas en inglés), la tasa fisiológica más extendida, pero no otros parámetros fisiológicos de gran relevancia en el estudio del nicho como pueden ser los umbrales de la zona termoneutra (TNZ, por sus siglas en inglés).

En cambio, en estudios longitudinales (aquellos en los que la especie o filogenia objeto se analiza a lo largo del tiempo) la disponibilidad de los rasgos para los escenarios temporales a comparar es la principal limitación, por ello, es habitual que incluyan los factores abióticos como rasgos asociados a las especies (ej.: Veloz *et al*., 2012; Maiorano *et al*., 2013). En este contexto la transferencia temporal de los SDMs, el análisis comparado del espacio ambiental o el estudio de la evolución de los rasgos a lo largo de las filogenias puede esclarecer las cuestiones sobre la conservación o evolución del nicho (Pearman *et al*., 2008; Cooper *et al*., 2011; Guisan *et al*., 2014). La transferibilidad temporal implica la habilidad que tiene un modelo calibrado en un periodo para ser aplicado de manera fiable en otro escenario temporal diferente (Tuanmu *et al*., 2011). La mayor restricción en la transferencia temporal de los modelos reside en la no analogía

en las condiciones climáticas de los escenarios temporales y/o espaciales comparados puesto que muchas especies pueden tener sus nichos truncados (Veloz *et al*., 2012). El truncamiento del nicho puede ocultar información sensible sobre las capacidades de tolerancia de la especie para afrontar las diferentes condiciones (Feeley & Silman, 2010), de ahí que pueda interferir en los resultados de las transferencias. En concreto, el truncamiento del nicho hace referencia a la adaptación de los taxones a condiciones abióticas del nicho, las cuales dejarían de estar disponibles en otras ventanas temporales y/o espaciales de dicho nicho (Guisan *et al*., 2014). Igualmente, la falta de equilibrio entre la distribución de la especie y el clima, que puede sesgar los resultados (Varela *et al*., 2009), de modo que es un punto crítico a considerar en el diseño del estudio. La falta de equilibrio en la distribución de la especie implica una ausencia de ésta en zonas que climáticamente le serían favorables pero, a causa de las interacciones bióticas o impedimentos en la dispersión, no puede ocupar (Araújo & Pearson, 2005). En los casos en los que existe equilibrio de la especie con el clima y el nicho no se encuentra truncado, la transferencia temporal es una metodología de gran utilidad pues permite identificar factores bióticos y/o abióticos responsables de alteraciones en la distribución de la especie a lo largo de su historia. Aunque, si bien, no ofrecen la posibilidad de discernir si los cambios en las distribuciones se deben a presiones externas o están promovidos por factores intrínsecos de las especies (como adaptación).

El análisis del espacio ambiental mediante técnicas de ordenación, elimina los posibles artefactos estadísticos asociados a los SDMs y minimiza ciertos errores que plantea el uso directo del espacio geográfico (Guisan *et al*., 2014; Di Cola *et al*., 2017). Bajo esta perspectiva se evalúa el solapamiento del nicho entre los diferentes escenarios temporales a lo largo de la historia de la especie, para ello se emplean directamente las presencias correspondientes a cada nicho y se comparan los cambios acontecidos en los respectivos marcos ambientales (ej.: Veloz *et al*., 2012; Maiorano *et al*., 2013). El análisis del solapamiento de nicho empleado en esta Tesis parte de la propuesta metodológica basada en los SDMs de Warren *et al*. (2008) y, posteriormente modificada por Broennimann *et al*. (2012) con una base de técnicas de ordenación. Originalmente Warren *et al*. (2008) plantearon un análisis para evaluar las diferencias de nicho a través de la comparación del espacio geográfico y tests de aleatorizaciones. A partir de esta concepción, Broennimann *et al*. (2012) formularon un nuevo marco estadístico con el que describir los nichos desde la perspectiva del espacio ambiental y en base a los tests de aleatorizaciones. La sustitución del espacio geográfico por el ambiental permite achacar las diferencias registradas a cambios en los nichos realizados de las especies y

no a peculiaridades entre las áreas comparadas, como diferencias en la disponibilidad relativa de ambientes dentro de los espacios geográficos (Broennimann *et al*., 2012).

Manteniendo el mismo enfoque, se puede implementar la evaluación de la conservación del nicho a lo largo de la filogenia integrando los factores abióticos del nicho como rasgos de las especies (ej.: Lv *et al*., 2016). La concepción subyacente a esta aproximación es que los nichos de las especies hermanas deben ser más similares (según PNC, mantendrían las condiciones del nicho ancestral) que los de las especies alejadas filogenéticamente (Cooper *et al*., 2011). De ahí que la comparación de los nichos entre especies hermanas debería rendir altos niveles de solapamiento. Una de las principales aportaciones de la consideración del componente espacial sobre los estudios filogenéticos recae en el esclarecimiento del origen de la similitud de los nichos, si bien es consecuencia de ancestros comunes o más bien refleja adaptaciones de las especies a condiciones similares (Cooper *et al*., 2011). Además, el empleo de las filogenias en el análisis del solapamiento del nicho permite una evaluación general de los taxones que supera las peculiaridades que pueden surgir con trabajos centrados en especies individuales sobre escalas regionales. Por tanto, las aportaciones que plantea esta combinación del espacio ambiental sobre las filogenias hacen que se trate de una metodología en auge el estudio de la PNC o PND (ej.: Aguirre-Gutiérrez *et al*., 2015; Rodrigues *et al*., 2019).

La subfamilia Arvicolinae (Mammalia, Cricetidae) constituye un excelente modelo de estudio para evaluar la conservación o evolución del nicho, por sus altas tasas de especiación en un reducido tiempo evolutivo (Triant & DeWoody, 2006). Dado que los procesos evolutivos promueven alteraciones en los nichos fundamentales (Pearman *et al*., 2008), esta alta tasa de especiación podría hacer que la falta de conservación de nicho rigiera entre los arvicolinos. Se estima que el origen del grupo podría remontarse a hace 6 millones de años (Charline *et al.*, 1999) y, en torno a 0.5 a 2 millones de años en géneros como los microtinos, y desde entonces la evolución en el genoma mitocondrial ha sido de las más rápida en comparación con otros linajes de mamíferos (Triant & DeWoody, 2006; Robovský *et al*., 2008). En consecuencia, los arvicolinos conforman uno de los grupos con más riqueza de especies entre los mamíferos (Martínková & Moravec, 2012).

Entre las especies de arvicolinos, consideramos relevante la relación con el clima de *Microtus cabrerae* (Rodentia, Cricetidae), una especie especialista del hábitat y endémica de la península Ibérica que presenta una distribución en contracción (Laplana & Sevilla, 2013). La historia evolutiva de *M. cabrerae* deriva de *M. brecciensis* durante el final del Pleistoceno Medio, que a su vez evolucionó de *M. huescarensis* al final del Pleistoceno Inferior (Laplana & Sevilla, 2013).

En base a peculiaridades morfológicas, genéticas (Jaarola *et al*., 2004), ecológicas y la historia evolutiva de la especie, Cuenca-Bescos *et al*. (2014) han propuesto que *Iberomys* sea elevado a la consideración de género. De este modo, *I. huescarensis*, *I. brecciensis* e *I. cabrerae* son las únicas especies que componen este género *Iberomys*, del que *M. cabrerae* es la única representante actual. Además por cuestiones de protección a la especie, Cuenca-Bescos *et al*. (2014) plantean que el nombre común de topillo de Cabrera sea sustituido por iberón, a fin de distinguirlos de otras especies de topillos que presentan altas densidades poblaciones y generan daños en los sistemas agrícolas. No obstante, las estudios genéticos más recientes (Barbosa *et al.*, 2018) no sustentan esta elevanción del subgénero a género y, por ende, abogan por el mantenimiento de la nomenclatura taxonómica de la especie como *Microtus cabrerae*.

M. cabrerae se caracteriza por ser un especialista de hábitats con una cobertura vegetal perenne a lo largo de todo el año y elevado nivel freático, que permite el mantenimiento de la vegetación a pesar de la de sequía estival. Se tratan de formaciones de berciales (*Stipa gigantea*), vallicares (*Agrostis castellana*) y fenalares (*Brachypodium phoenicoides*) en combinación con juncales mediterráneos (*Scirpus holoschoenus*) (San Miguel, 1994), que les proporcionan alimento y cobijo, ya que es una especie de comportamiento epigeo. Su distribución sigue un arco que abarca el suroeste, centro y sureste peninsular y áreas aisladas en el sistema prepirenaico, según cuatro núcleos: Luso-carpetano, Bético, Montibérico y Prepirenaico (Garrido-García *et al*., 2013) (Figura 2).

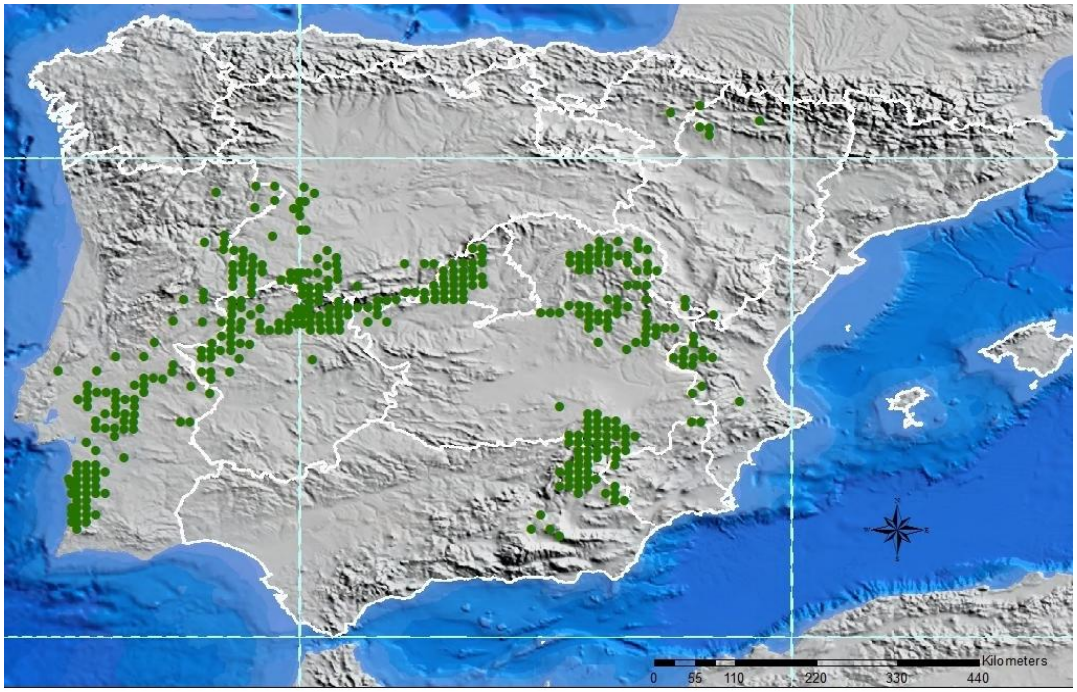

Figura 2. Distribución mundial de *Microtus cabrerae*.

La especie se encuentra catalogada como “próxima a la amenaza” a nivel internacional según la Lista Roja de Especies Amenazadas (IUCN) (Fernandes *et al*., 2008); mientras que en el territorio español se le considera “vulnerable” por su escasa y fragmentada distribución (Fernández-Salvador, 2007). La mayor amenaza que se cierne sobre *M. cabrerae* es la destrucción de su hábitat a causa del aprovechamiento ganadero, agrícola y la construcción de infraestructuras (Fernández-Salvador, 1998, 2007).

En suma, ante la alta tasa de especiación del grupo, resulta previsible que se hayan podido acumular numerosas variaciones genéticas susceptibles a promover cambios en el nicho, de modo que la conservación del nicho sería especialmente improbable. Por otro lado, dado que es un grupo con marcadas preferencias tróficas y de hábitat, a pesar de labilidad genética, algunos componentes básicos del nicho podrían haberse mantenido a lo largo del proceso evolutivo. Por todo esto y la vulnerabilidad de la especie frente a las condiciones de cambio climático, pensamos que *M. cabrerae* es un buen modelo en el estudio de la conservación del nicho. Además, consideramos que los resultados derivados de esta Tesis pueden tener una elevada funcionalidad en una especie de la que se conoce tan poco. A través de los distintos enfoques metodológicos podremos esclarecer las respuestas metabólicas, históricas y filogenéticas de la especie, información de gran utilidad con la que nutrir los programas de protección de la especie.

Objetivos

El objetivo general que se persigue en esta Tesis es la identificación de los patrones que señalan hacia la conservación o la evolución del nicho de *M. cabrerae*. Con este fin, se emplea un enfoque inicial experimental (Capítulos 1 y 2) y un posterior análisis basado en el modelado (Capítulos 3 y 4). A través de la parte experimental se persigue evaluar la conservación o la evolución del nicho a corto plazo (escala temporal de individuo) empleando la ecofisiología, es decir, se trata de registrar los cambios que ocurren sobre los rasgos fisiológicos en respuesta a los cambios abióticos del hábitat. En cambio los capítulos basados en el modelado consideran una amplia escala temporal, se tratan de estudios longitudinales que evalúan los cambios en el nicho de la especie a lo largo de su historia o incluso a lo largo de la filogenia. La combinación de ambos enfoques permite obtener una perspectiva global de las capacidades y limitaciones de la especie no sólo como respuesta a los cambios actuales, sino también en base a su historia evolutiva. De este modo, las conclusiones sobre la conservación o la evolución del nicho de *M. cabrerae* se extraen de un amplio abanico de evidencias que relacionan la respuesta de la especie y sus congéneres a las alteraciones abióticas. En consecuencia, los objetivos específicos que articulan esta Tesis serán los siguientes:

- Determinar los rasgos fisiológicos que definen a la especie en relación con las condiciones abióticas. Para ello se medirán los principales parámetros fisiológicos (BMR y TNZ) de la especie, ya que sólo el BMR ha sido determinado anteriormente en las poblaciones portuguesas (Mathias *et al*., 2003). Además, se evaluará la variabilidad intraespecífica en los parámetros fisiológicos según la distribución de la especie a lo largo de los pisos bioclimáticos supra- y mesomediterráneos. Estos pisos determinan un régimen climático y de recursos que potencialmente pueden afectar los requerimientos energéticos de los individuos y, con ello, a sus rasgos metabólicos (Capítulo 1, Castellanos-Frías *et al*., 2015).
- Estudiar la capacidad de plasticidad fenotípica y adaptativa de la especie a través de los rasgos fisiológicos ante cambios en las condiciones abióticas. Se pretende determinar si la especie presenta una respuesta plástica a alteraciones en las condiciones abióticas, por lo que se planteará un experimento de jardín común en que se someterá a los individuos a alteraciones en el ambiente térmico controlando el resto de factores (humedad, alimentación, comportamiento, etc.) (Capítulo 2).
- Evaluar los posibles cambios en el nicho de la especie a lo largo de su historia (desde el Pleistoceno Superior hasta la actualidad). Con este fin se seleccionarán diversos escenarios climáticos contrastados (Último Interglacial, Último Máximo Glacial o LGM y el

Holoceno Medio) junto con sus correspondientes registros fósiles de la especie. Se aplicará una doble metodología basada en la proyección temporal de modelos de distribución de especies (análisis del espacio geográfico) y la comparación del espacio ambiental ocupado por el nicho de la especie en cada escenario temporal (análisis del espacio ambiental). Ambas aproximaciones proporcionarán diversos índices estadísticos para la evaluación cuantitativa de la conservación o evolución del nicho (Capítulo 3, Castellanos-Frías *et al*., 2018).

- Identificar los patrones de conservación o evolución del nicho a lo largo de la filogenia de los arvicolinos, con especial atención a las variables bioclimáticas de los nichos realizados de las especies. A tal fin, se mapearán las variables de interés en un árbol filogenético de consenso de los arvicolinos de la región Holártica, incluyendo grupos con distintas estrategias vitales, hábitats o tamaños. A través de una metodología basada en el análisis del espacio ambiental, se compararán los nichos realizados de las especies hermanas para establecer el grado de conservación del nicho en este amplio grupo de mamíferos (Capítulo 4).

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Listado de manuscritos

Esta Tesis Doctoral se articula en torno a 4 artículos escritos en inglés para su publicación en revistas científicas de carácter internacional e indexadas (SCI). A continuación, se detalla el listado de coautores, el título y el estado de publicación.

Capítulo 1. Castellanos-Frías, E., García-Perea, R., Gisbert, J., Bozinovic, F., Virgós, E. 2015. Intraspecific variation in the energetics of the Cabrera vole. *Comparative Biochemistry and Physiology, Part A.* 190: 32-38.

Capítulo 2. Castellanos-Frías, E., Bozinovic, F., Virgós, E. The intraspecific variability in the energetics of a habitat-specialist species: a common garden approach to study phenotypic plasticity and adaptation. Manuscrito en preparación.

Capítulo 3. Castellanos-Frías, E., García, N, Virgós, E. 2018. Assessment of the effect of climate changes in the Late Pleistocene and Holocene on niche conservatism of an arvicolid specialist. *Scientific Reports*. 8: 1-11.

Capítulo 4. Castellanos-Frías, E., García, N, Virgós, E. Is the niche preserved in the Arvicoline species from the Holarctic? Manuscrito en preparación.

Capítulo 1

Intraspecific variation in the energetics of the Cabrera vole

Castellanos-Frías, E., García-Perea, R., Gisbert, J., Bozinovic, F., Virgós, E.

Manuscrito publicado en: *Comparative Biochemistry and Physiology, Part A.* 190: 32-38.

Abstract

Basal metabolic rate (BMR) is an intensively topic studied in ecophysiology for the purpose of understanding energy budgets of the species, variations of energy expenditure during their diary activities and physiological acclimatization to the environment. Establishing how the metabolism is assembled to the environment can provide valuable data to improve conservation strategies of endangered species. In this sense, metabolic differences associated to habitats have been widely reported in the interspecific level, however little is known about the intraspecific view of BMR under an environmental gradient. In this study, we researched the effect of the habitat on metabolic rate of an Iberian endemic species: *Microtus cabrerae*.

Animals were captured in different subpopulations of its altitudinal range and their MR was studied over a thermal gradient. MR was analyzed through a Linear Mixed Models (LMM) in which, in addition to thermal effects, the bioclimatic zone and sex also influenced in the metabolism of the species. The beginning of thermoneutrality zone was set on 26.5° C and RMR was 2.3 ml $O_2 \cdot g^{-1} \cdot h^{-1}$, intermediate between both bioclimatic zones. Supramediterranean subpopulations started the Tlc earlier (24.9° C) and had higher RMR than the mesomediterranean ones (26.9° C). The thermal environment together with primary productivity conditions could explain this difference in the metabolic behaviour of the Cabrera voles.

Key words. Cabrera vole; Ecophysiology; Global change; *Microtus cabrerae*; Intraspecific variability; Oxygen consumption; RMR; Thermoregulation; TNZ.

Introduction

Macrophysiological patterns and its variability in time and space are of vital importance to gain insight about evolutionary and ecological theories (Bozinovic *et al*., 2014; Chown and Gaston, 2008; Naya *et al*., 2013). In addition, the relatively recent trend in niche modelling literature has evolved from correlative models relating presence of species to niche conditions to mechanistic models based on physiological data (Kearney and Porter, 2009). Despite the correlative approach is widely used (*e.g.* Anderson and Raza, 2010; Zeng *et al*., 2015) it shows low predictive power when is used to predict abundance and distribution over novel environmental conditions and it cannot explain the fundamental causation of geographical distribution (Kearney and Porter, 2004), which could be satisfied with the mechanistic approach. Mechanistic models integrate behavioural, morphological and physiological traits of the organism with the habitat features using energy and mass balance equations to determinate the species' range (Barve *et al*., 2014). In this field, the energetic metabolism is an intensively studied topic (*e.g.* Agosta *et al*., 2013; Rezende *et al*., 2004), owing to some of its traits as the metabolic rate responses directly to climate conditions which make that it can be related to the fundamental niche and then to be used to model distribution and abundance or predicting potential changes linked to novel climate conditions.

Among various physiological measurements of ecological relevance, basal metabolic rate (BMR) represents the minimum rate of energy necessary to maintain homeostasis and allostasis. BMR has been used as a standard to assess the costs of different components of organism energy budgets, to analyze species-specific as well as intraspecific variations in energy expenditure during maximal and sustained activities, and to understand physiological adaptations to the environment (McNab, 2002). The dependence of metabolic rates on body mass (M_b) has long been recognized (Kleiber, 1961). Nevertheless, M_b alone does not fully explain variation in BMR (McNab, 1992). There are several hypotheses that attempt to explain how biotic and abiotic conditions affect mass-independent BMR in mammals (McNab, 2002), namely, food quality, food availability and/or unpredictable, direct climate effects, aridity, among others. For instance, several interspecific studies have analyzed the effects of food availability and predictability on mass-independent BMR by demonstrating that mass-independent BMR is higher in mesic habitats when compared with xeric habitats (Bozinovic *et al*., 2007; Lovegrove, 2000; McNab, 2002; Mueller and Diamond, 2001; Rezende *et al*., 2004). Among rodent species it has been largely reported the clear association between the mass corrected-metabolism rate and several key abiotic factors, such as: latitude (Lovegrove, 2003; Rezende *et al*., 2004; Speakman, 2000),

altitude (Hayes, 1989), or climate (Bozinovic and Rosenmann, 1989; Hulbert *et al*., 1985; McNab, 1970). Most of these studies evaluated the effect of the environment on BMR from an interspecific level approach, considering that the traits are fixed and thus the BMR value is unique for each species. Nevertheless, some genetic or plastic variability can exist so the analysis of the metabolism rates focus on the intraspecific level allows to approximate the factors responsible for variations in BMR and the ability of the species to adapt to the environment (Cruz-Neto and Bozinovic, 2004). Therefore, studies on changes in BMR across environmental gradients can help to predict the potential response of the species to anthropogenic changes in the environment and then to design better strategies for their conservation.

We investigated here the effects of geographic variations in habitat on the energetics (*i.e.* RMR, minimal thermal conductance (Cm) and minimal temperature differential between body (Tb) and environment (Ta) = ΔTm) of rodents originated from different populations. We used as study model populations of *Microtus cabrerae* which is an endemic vole of the Iberian Peninsula, classified as Near Threatened in the International Union for Conservation of Nature (IUCN) Red List of Threatened Species (Fernandes *et al*., 2008). The species has a highly fractured distribution area that is restricted to the centre, southwest and southeast of the Peninsula (Garrido-García *et al*., 2013). The Cabrera vole is a specialist species of scenopoetic variables with very restrictive habitat preferences. It has a patchy distribution in open areas with high water table and covered by formations of perennial grasses and rush beds. The subpopulations are distributed along an altitudinal range from 250 to 1500 m, although they are more common in meso- and supramediterranean bioclimatic zones, between 500 and 1200 m (Fernández-Salvador, 1998). Because of its habitat specificity and limited geographical distribution, the species is highly vulnerable to anthropic activities such as agriculture, farming or human infrastructures (Landete-Castillejos *et al*., 2000). Furthermore, predictions on climate change in the Iberian Peninsula expect an increase in the xericity (Pachauri *et al*., 2014) which could change the suitability of Cabrera voles' habitats compromising its conservation and persistence.

Mathias *et al*. (2003) and Santos *et al*. (2004) have explored the energetic metabolism of *M. cabrerae* but ignoring the intraspecific approach of this physiological trait. Consequently, our main aim is to evaluate the energetics of Cabrera vole at an intraspecific level and to test for interpopulation differences associated to bioclimatic conditions.

Materials and methods

Experimental animals

This study is made up of 15 adults of *M. cabrerae*, from different subpopulations of two bioclimatic zones: mesomediterranean (subpopulations between 480 m and 710 m a.s.l.) and supramediterranean (subpopulations between 900 m and 1050 m a.s.l.) in Madrid, Spain. (Fig. 1). A total of 8 animals were captured for mesomediterranean bioclimatic zone (5 females and 3 males) and 7 in the supramediterranean (4 females and 3 males). Cabrera voles were captured using Sherman live traps supplied with apples, during the day because of this species shows diurnal and nocturnal activity (Fernández-Salvador, 1998). Due to the randomness of trapping, it was not possible to obtain equal number of male and female from each bioclimatic range. *M. cabrerae* is a Near Threatened species (Fernandes *et al*., 2008), thus we had to reach a compromise between selecting a representative number of voles and not affecting negatively to the sampled subpopulations, so we had to use a reduced sample of experimental animals.

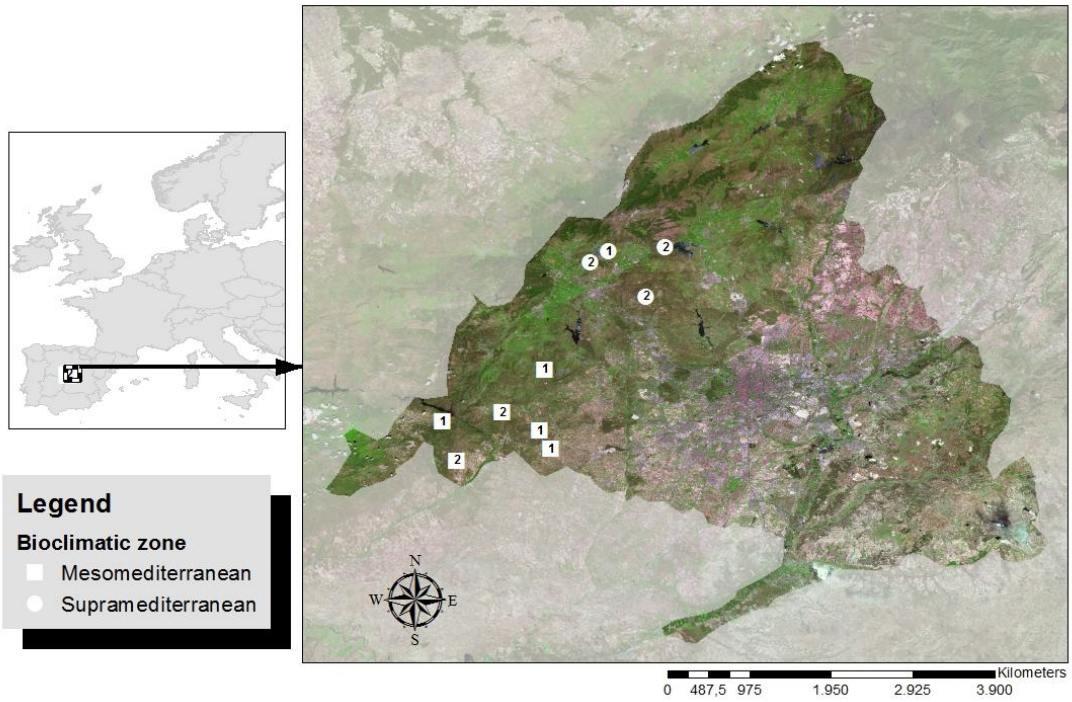

Figure 1. Geographical localization of the subpopulations where the Cabrera voles were taken for the study. Numbers in the symbol are individuals captured in the respective subpopulation.

Trapping were conducted in four different subpopulations in the supramediterranean zone and in six subpopulations in the mesomediterranean, during June and July of 2013 (Fig. 1). The annual average temperature in subpopulations of the supramediterranean zone varies between 11.1° and 11.9° C; with maximum temperatures in July between 21.2° and 21.9° C; minimum temperatures in January ranging from 2.8° to 3.4° C; the minimum rainfall values occur in august (14 and 15 mm); and total rainfalls of the area varies from 472 to 484 mm. Whereas in the subpopulations of the mesomediterranean zone the annual average temperature varies between 13° to 14.6° C; the maximum temperatures occur in July with values from 23.3° to 24.9° C; and minimum temperatures in January, ranging from 4.4° to 5.7° C; the minimal rainfalls happen in July, from 9 to 12 mm; and total rainfalls ranging from 370 to 419 mm (AEMET, 2015). These climate differences between bioclimatic zones involve differences in the configuration and composition of the vegetation of the colonies. Supramediterranean subpopulations are large areas made by dense structures of *Stipa gigantea*, and sometimes they also include patches of *Agrostis castellana*. However, in the mesomediterranean range subpopulations occupy smaller areas with a continuous canopy of *Agrostis castellana* and scattered rushes (*Juncus* spp.).

The effect on primary productivity is a higher averaged value of the Normalized Difference Vegetation Index (NDVI), a proxy of primary productivity (Pettorelli *et al*., 2005), for supramediterranean areas, although the difference between areas were statistically marginal (ANOVA: $F_{1,8}$=4.65; p=0.06), we observed ecological differences between habitats. The NDVI values for subpopulations were derived from MOD13Q1 NDVI, a satellite image captured by the MODIS sensor aboard the NASA's TERRA satellite (https://lpdaac.usgs.gov/products/) and processed with ArcGIS tools. Only one satellite image was downloaded corresponding to the voles' capture date (June of 2013), with a spatial resolution of 231 m x 231 m. This is the minimum spatial resolution image that allowed us to cover the NDVI variability for the size of the subpopulations evaluated (from 500 m^2 to 47000 m^2).

Each captured animal was transported to the laboratory on the same day of capture and its metabolism rate was measured within 24 hours. The animals were distributed in individual transparent cages (40x26x27 cm) accommodated with hay to simulate vole's habitat and fed with apple and oat *ad libitum* (Fernández-Salvador *et al*., 2001). Finally, all voles were released alive in their respective subpopulations. All animal procedures were approved by the Animal Ethics Committee of The Rey Juan Carlos University of Madrid (Spain).

Metabolic rates

Metabolic rate of *M. cabrerae* was measured as the rate of oxygen consumption (VO_2), using a computerized open-flow respirometry system (Sable Systems, Anderson, NV). Animals were situated inside a metabolic chamber (volume, 1.3 L) that was provided with a metallic grid which supports the voles to isolate them from their own faeces and allows them to adopt a relaxed posture during measurements. The size of the chamber was a compromise between ensure some animal movement but small enough to prevent excessive activity. The metabolic chamber was pumped with a flow of 800 ml·min^{-1} of dried atmospheric air which is enough to ensure the mixture inside. On the entry and the outlet of the metabolic chamber were placed a CO_2–absorbent filter of Soda Lime and a H_2O-absorbent filter of Drierite, arranged in series, so avoiding the CO_2 and H_2O influence in the O_2 measures. The output flow was sampled each 5 seconds by the FoxBox Oxygen Analysis System (Sable Systems, Anderson, NV), and the oxygen consumption was analyzed with the ExpeData data acquisition software (http://www.sablesys.com) following the equation of Withers (1977):

$$Vo_2 = \frac{V_E \cdot (FI_{O2} - FE_{O2})}{1 - FI_{O2}}$$

Where VO_2 is the rate of O_2 consumption; V_E is the rate of airflow pumped into the metabolic chamber; FIo_2 is the fractional concentration of O_2 entering the metabolic rate; and FEo_2 is the fractional concentration of O_2 leaving the metabolic rate. Cabrera voles were measured at different ambient temperatures ranging from 5° to 36° C (*ca.* 4 hours). At each temperature the metabolic rate was taken as the minimal oxygen consumption at least during 3 minutes. Metabolic rates were expressed as mass-specific metabolic rates (ml $O_2 \cdot g^{-1} \cdot h^{-1}$). Since *M. cabrerae* is a near threatened species we did not want to jeopardize it, so we delimited the maximum temperature at which submit voles in 36° C. Within the thermoneutrality range, the metabolic rates were measured as resting metabolic rates (RMR) because animals were not under fasting conditions. Minimal thermal conductance (Cm) was calculated as the slope of the metabolism-environmental temperature curve under cool conditions (ml $O_2 \cdot g^{-1} \cdot h^{-1} \cdot {}^{o}C^{-1}$). Voles were weighted with an electronic scale (±0.1 g) before and after VO_2 records.

Resting metabolic rate was compared with the predicted standard rate of metabolism for eutheria: BMR = $3.42 \cdot M_b^{-0.25}$ (Kleiber, 1961), small grazers: BMR = $7.65 \cdot M_b^{-0.38}$ and BMR =

$4.18 \cdot M_b^{-0.32}$ (McNab, 1988). In addition we compared the observed Cm against the expected one based on $Cm = 1.0 \cdot M_b^{-0.50}$ (Morrison and Ryser, 1951). According to Cooper and Withers (2006) the 95% prediction confidence intervals were calculate for each one of these allometric regressions. We followed this approach to evaluate statistically if the Cabrera vole's energetic traits (RMR and Cm) were closed to the expected values based on M_b. The minimal (body-ambient) thermal differential (ΔTm) was calculated in each habitat and was also used to estimate the theoretical lower critical temperature of thermoneutrality (Tlc^*) for the species following the McNab's (1974) equation ($\Delta Tm = Tb\text{-}Tlc = BMR/Cm$ or $\Delta Tm = Tb\text{-}Tlc = 3.42 \cdot M_b^{0.25}$).

Statistical analysis

All statistical analysis were performance in the R software (R Core Team, 2014) through suitable packages for linear mixed models (LMM): nlme (Pinheiro *et al*., 2014) and lme4 (Bates *et al*., 2014) with normal response distribution for metabolic rate variable. To evaluate the variability over the metabolic rate we design a model where sex, bioclimatic zone, temperature and individuals were tested. The model was composed by the metabolic rate as the response variable with continuous range, the bioclimatic zone and sex were fixed factors with two levels, respectively, as environmental temperature was also a fixed factor with 12 levels to cover the range from 5° to 36° C. Voles were included in the model as random effects to control individual conditions and to generalize the results to Cabrera vole's populations. The structure of random effects were optimized following the protocol outlined in Zuur *et al*. (2009), considering the contribution of fixed factors to modify the intercept and/or slopes of functional relationship between RMR and individuals. According to Bolker *et al*. (2009) and Zuur *et al*. (2009) in each model the parameter estimation of fixed effects was done under maximum likelihood (ML) method, while the final model estimation of the parameters which better described the metabolic response of *M. cabrerae* was based on restricted maximum likelihood (REML). Thus, it was designed a battery of 19 models in which the goodness of fit to the dataset was assessed with the corrected Akaike Information Criterion (AICc). Better models were selected from the model set considering as good models they which did not differ in more than 2 units of AICc (ΔAICc) from the model with the lower AICc (Burnham and Anderson, 2002) thanks to the functions of the package MuMIn (Barton, 2014). *Model average* function from MuMIn package was used to get the only one best model, so its model coefficients were averaged according to the relative importance of each independent variable and the number of models containing

those variables. Finally the structure of the residuals was evaluated to corroborate the good fit of the selected model to the dataset.

The identification of the thermoneutrality zone was conducted based on piecewise regression thresholds to reveal the point of change. Piecewise regressions are widely employed for modelling ecological relationships defined by abrupt changes in the response variable when an ecological threshold is exceeded (Ficetola and Denoël, 2009; Rhodes *et al*., 2008). The beginning of thermoneutral zone implies a change in the slope of the regression curve of metabolic rate, thus the piecewise regression threshold analysis was focused on finding a relevant change in that slope. Intervals for the breaking points of the piecewise regression threshold were defined inside the linear mixed model with the function *lmer*.

Results

The metabolic response of the *M. cabrerae* to the range of their environmental temperatures was explained through a linear mixed model that included temperature, sex and bioclimatic zone as fixed factors and a random effect over the individual with a variable effect depending on the temperature. Only one better model was chosen to explain the metabolism differences in the subpopulations of Cabrera vole (Table 1). The selected model explained the 88% of the data variance, in which 77% was assumed by fixed factor and only the 11% of the variance was as consequence of individuals.

Table 1. Summary of the four best linear mixed models to explain the metabolic rate of Cabrera voles. In bold is highlighted the best model that was the only selected model.

Model	T	BZ	S	BZ:S	S:T	*k*	logLik	ΔAICc	w_i	R^2m	R^2c
1	**X**	**X**	**X**	**X**		**5**	**-143.14**	**0.00**	**0.39**	**0.77**	**0.88**
2	X	X	X			4	-144.52	0.53	0.30		
3	X		X			3	-146.27	1.83	0.16		
4	X	X	X	X	X	6	-143.00	1.99	0.15		
Parameter weights	*1.00*	*0.84*	*1.00*	*0.54*	*0.15*						

k is the number of parameters in the model; ΔAICc is the difference in AICc between each model with the best model (with lower AIC); w_i is the AICc weight of the average model. The fixed parameters in the model are included by their initials (T: temperature; BZ: Bioclimatic zone; S: Sex). The R^2c is the conditional R^2 which is referred to the global model while R^2m is the marginal R^2 that is only bounded to fixed effects of the model. These last parameters were only expressed for the selected model. *Parameter weights* reveal the relative importance of each variable depending on the number of containing models in which they are included after analyzing the models with the model average function.

The relationship between MR and temperature followed the general trend of endothermic homeotherms animals with a change rate of -0.126 and a random variance of 0.001 associated to the individual response while the temperature increased (Table 2).

Table 2. Parameters estimated by the selected model to explain the metabolic response of *M. cabrerae*

Fixed effects						Random effects			
	Intercept	T	BZs	Sm	BZs:Sm		Individual	T	Residual
Estimate	6.217	-0.126	0.509	-0.348	-0.381	**Variance**	0.607	0.001	0.254
SE	0.284	0.008	0.332	0.319	0.448	**SD**	0.779	0.027	0.504

T is the temperature factor, BZ is the bioclimatic zone and S is the sex, BZ:S indicate the interaction between both factors. In the model's output the estimate summaries the mesomediterranean level of the factor Bioclimatic zone (BZ), the female level of the factor Sex (S) is also included in the intercept, as well as the interaction between the levels mesomediterranean and female sex. Thus, the fixed factor bioclimatic zone only expressed the estimate for the supramediterranean level (BZs), the sex factor only included the parameters for males (Sm) while the interaction between both factors considered the same levels (BZs:Sm).

Under the whole temperature cycle, the average of metabolic rate was higher for supramediterranean voles than mesomediterranean (Fig. 2). The model estimates a metabolic rate for mesomediterranean zone that is summarized in the intercept value (Table 2) over which there is an increment of 0.509 (± 0.332) for explaining the metabolic rate of supramediterranean zone.

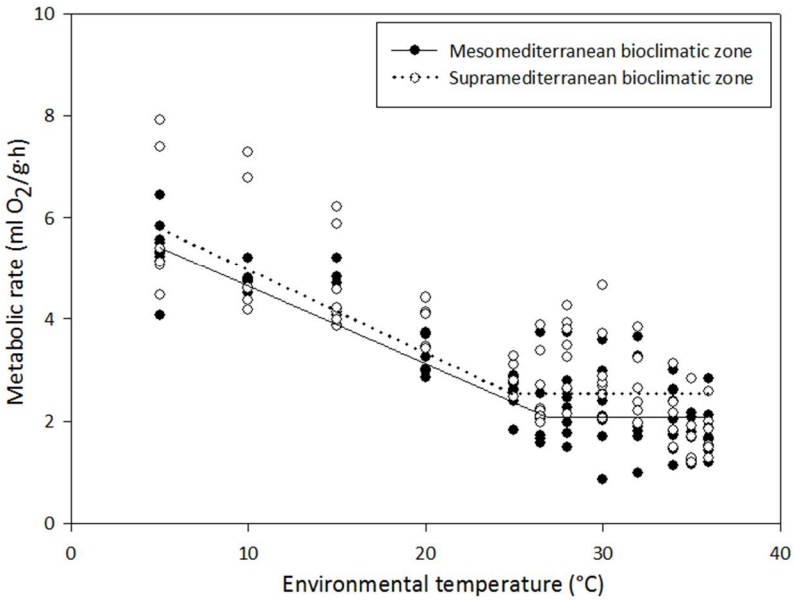

Figure 2. Individual metabolic rate of *M. cabrerae* from different bioclimatic zones in response to ambient temperatures. The change in the slope of the curve reveals the beginning of the thermoneutral zone for each bioclimatic zone.

Sex differences had also influence over the metabolic rate voles studied. Males from both bioclimatic zones had lower metabolic rates, in average they had a value of -0.348 (± 0.319) over the intercept (Table 2). In contrast, females had upper rates, which increased in 0.509 (± 0.332) for females from supramediterranean zone and just the intercept value for mesomediterranean zone females.

The individual metabolic response of *M. cabrerae* to the range of ambient temperatures allowed defining the zone of thermoneutrality for the species (Fig. 2). At low ambient temperatures the metabolic rate was high and decreased as the temperature increased until 26.5° C when the rate tended to the stability and thus TNZ started then. In the temperature range above the starting point of TNZ, the metabolic rate tended to the resting metabolic rate. Metabolic variables for subpopulations of each Bioclimatic zone studied are compiled in Table 3. The upper limit of the TNZ could not be determined to avoid any death.

Table 3. Body mass (M_b), resting metabolic rate (RMR), lower critical temperature of thermoneutrality (Tlc), theoretical lower critical temperature of thermoneutrality (Tlc^*), thermal conductance (Cm) and minimal (body-ambient) thermal differential (ΔTm) for the global species and the subpopulations of *M. cabrerae*.

	M_b (g)		RMR (ml O_2/g·h)		Tlc (°C)	Tlc^* (°C)	**Cm** (ml $O_2 \cdot g^{-1} \cdot h^{-1} \cdot {}^oC^{-1}$)	ΔTm (°C)	% BMR	% BMR (small grazers)	% BMR (order Rodentia)	**% Cm**
	mean	sd	mean	sd					Kleiber (1961)	McNab (1988)		Morrison & Ryser (1951)
M. cabrerae	51.16	6.09	2.278	0.797	26.49	27.36	0.142	15.9	77.8%	34.4%	91.2%	10.9%
Supramediterranean subpopulation	51.19	5.52	2.539	0.819	24.85		0.138	18.4	98.2%	49.8%	113.1%	7.7%
Mesomediterranean subpopulation	51.14	6.58	2.078	0.694	26.85		0.146	14.2	62.2%	22.6%	74.4%	13.8%

The percentages represent the increase over the theoretical values.

The percentage of change of metabolic traits measured in our study in comparison to the predicted values from the allometric equations of Kleiber (1961), McNab (1988) and Morrison and Ryser (1951) are summarized in Table 3. The Cabrera vole's RMR for the global species and the analyzed by bioclimatic zones were higher than the expected from M_b and also fell out the 95% of the predicted confidence intervals for the three allometric regression (1.241-1.549 (Kleiber, 1961); 1.466-1.956 and 1.117-1.253, for small grazer mammals and Rodentia mammals, respectively (McNab,1988)). Also, and regarding thermal conductance, values of the species and subpopulations were higher than those predicted from the mass (Morrison and Ryser, 1951) and were outside the 95% predicted confidence interval (0.120-0.137).

Discussion

Levels of metabolic rates in mammals are influenced by different factors, including body mass, phylogenetic relatedness, activity, biotic and abiotic habitat conditions, and food habits among others (McNab, 2002). For example, Mueller and Diamond (2001) and Bozinovic *et al*. (2007) found a high-BMR in rodent species and populations from high-productivity environments in comparison to species and populations from low-productivity habitats. These authors concluded that BMR is driven by NPP and, hence, food availability.

Cabrera vole is a robust species with higher body mass than similar voles of the genus *Microtus* (Fernández-Salvador, 1998). Despite of *M. cabrerae* are relatively heavy voles, its mass-specific RMR values are relatively lower than the values reported for other arvicolines species (Cricetidae, Rodentia) such as *Chionomys nivalis* (35 g), *Microtus agrestis* (22 g) (McNab, 1986), *M. arvalis* (30 g) (Hart, 1971), *M. oeconomus* (32 g) (Lantova *et al*., 2011), *M. maximowiczii* (38 g) (Chen *et al*., 2012) or *Clethrionomys glareolus* (23 g) (Aalto *et al*., 1993; Labocha *et al*., 2004). However, average values obtained in this study (2.278 ± 0.797 ml $O2 \cdot g^{-1} \cdot h^{-1}$) are higher than the ones reported by Mathias *et al*. (2003) for the same species but situated in lower altitudes in Portugal. In addition to the altitude differences, the Atlantic influence on climatic conditions and grass vegetation of the Portuguese populations could produce particular habitats which reduce the metabolic rate, maybe implying lower habitat quality for this population (*e.g.* food quality hypothesis, Bozinovic *et al*., 2007; Bozinovic *et al*., 2009).

Against the theoretical metabolism predicted by Kleiber (1961), our results reveal a higher resting metabolic rate which underscores that the metabolism is also contributed by ecological factors (Luna *et al*., 2009; Rezende *et al*., 2004). Alike, our outcomes exceed the BMR predicted

for small grazer mammals or for mammals from order Rodentia (McNab, 1988). Small grazer mammals as microtines have higher basal metabolic rates than the expected for their body mass, as consequence of their food habits (grazing evergreen food), their terrestrial behaviour that requires high levels of energy to escape from predators or as result of the climate in their habitats (McNab, 1986). Furthermore, the BMR level is also contributed by the taxonomic affiliation (Hayssen and Lacy, 1985) so, the BMR averaged of species from genus *Microtus* is a 82.2% higher than the BMR expected for species of order Rodentia (McNab, 1988), hence that an exceeded level of RMR over the predicted was expected for Cabrera voles.

In the same way, the thermoneutrality zone defined for *M. cabrerae* in our study ranges from 26.5° to 36° C, which comes early the beginning of TNZ with respect of the 30° C identified by Mathias *et al*. (2003) for this species. Differences between both values are attributed to local conditions of the analysis which makes that the TNZ cannot be extrapolate to any environment (Romanovsky *et al*., 2002) or because TNZ can be affected mainly by differences in habitat quality, productivity or climate. The maximum threshold of measured temperature was 36° C, for avoiding any animal dead, hence we cannot determine the upper limit of the thermoneutral zone; however we suppose that it is closed to the upper critical temperature of TNZ, because upper lethal temperature is often 3°- 6° C above the normal body temperature (36.6° C, Castellanos-Frías *et al*., unpublished data) (Willmer *et al*., 2005). The Tlc is closed to the theoretical Tlc* which situates the beginning of TNZ in 27.36° C, only 0.9° C warmer than the measured in this study. Our results support data from other arvicolines species, the Cabrera vole's TNZ covers a slightly warmer and wider temperature range than that of other species as *M. agrestis* from 25° to 30° C (McDevitt and Speakman, 1994), *M. maximowiczii* from 25° to 32.5° C (Chen *et al*., 2012) or *Clethrionomys glareolus* with a range from 25° to 30° C (Aalto *et al*., 1993). The intraspecific analysis reveal an earlier beginning in TNZ for supra-populations (Tlc = 24.85° C) than in mesomediterranean (Tlc = 26.85° C), this pattern of advance in temperature of TNZ might be related to colder thermal environments (Willmer *et al*., 2005).

In general, the low value of resting metabolic rate could manifest a selective advantage of low metabolic rates in habitats with low-medium trophic quality (Louw and Seely, 1982), because the minimal energetic requirements are reduced (Veloso and Bozinovic, 1993) and then animals can survive with lower food supply. Summer conditions in Mediterranean areas define food restrictions and unpredictability in Cabrera voles' habitats. Droughts joined high temperatures promote the reduction of trophic resources available (*e.g.* green grasses, Bozinovic *et al*., 2009), thus summers act as a bottleneck regulating their populations and selecting towards minimal

rates. Ventura *et al*. (1998) and Mathias *et al*. (2003) suggested that there could be a seasonal different response in body mass and basal metabolic rate of *M. cabrerae*, however this study has not considered that variance. The aridity increase of Mediterranean climate is one of the main short time threat with which this species has to face to, thus we studied the restrictive period in the species and focused on spring-summer when the climatic conditions could question the survival of the individuals (Fernández-Salvador *et al*., 2005).

Metabolic differences assigned to altitude could be mainly attributable to the effect of the thermal environment and the primary production of habitat, since the difference of altitudinal is not large enough to account for other effects (*e.g.* Po_2 or radiation load). The thermal environment contributes significantly to the variance in metabolic rate between species, thus Bozinovic and Rosenmann (1989) found that the cold-induced maximum metabolic rate (MMR) was higher in rodents from cold climates, while tropical species showed tendency to lower values. Under low temperatures, the metabolic rates of the endothermic animals increase as consequence of their thermoregulatory costs (McNab, 2002), hence the predominance of cooler conditions at higher altitudes arises as the basis for differences in RMR between supra- and mesomediterranean subpopulations. Along the same lines, the strategies to keep or dissipate the heat are adapted to the thermal environment (Willmer *et al*., 2005), nevertheless, in spite of the thermal contrast in the areas of study, no major differences occur in the conductance value between individuals from both bioclimatic zones. The same pattern is kept in the species' conductance that is slightly lower than Mathias' *et al*. (2003) average (0.152 ± 0.008 ml $O_2 \cdot g^{-1} \cdot h^{-1} \cdot ° C^{-1}$). The robustness in the magnitude of thermal conductance is an expected result, since it depends mainly on the body mass (Morrison and Ryser, 1951) and there is not body weight differences between samples of both studies, little differences are due to the temperature differential between the animal and its environment. Thus, compared to the theoretical conductance, our outcome is lightly higher (11%) than the predicted value for this species. A review by bioclimatic levels reveals that mesomediterranean subpopulations have a thermal conductance higher than the supramediterranean ones, reflecting the greater need of meso-subpopulations to dissipate heat. Consequently, ΔTm is wider for supra- than mesomediterranean subpopulation, hence supramediterranean individuals have a little wider range of thermal tolerance for which the metabolic rate is the minimum, and thus advantageous for this subpopulation.

Regarding to primary production of habitats, both bioclimatic zones differ in the vegetation composition, which could contribute to differences between bioclimatic zones, so the

supramediterranean subpopulations were mainly constituted by *S. gigantea* and sometimes by *A. castellana* while the mesomediterranean only had *A. castellana*. According to the *Food Quality Hypothesis* (Bozinovic *et al*., 2007; Bozinovic *et al*., 2009; Cruz-Neto and Bozinovic, 2004) BMR values are associated with the diet, thus the variability of habitats in food quality, availability and predictability leads to differences in BMR into the species. In spite of the nutritional component of habitats was not evaluated in the study, however values of NDVI for summer period showed slighter higher productivity in the supramediterranean than in mesomediterranean range, this difference was marginally non significant statistically. Because the number of subpopulations sampled was small to compare NDVI values between bioclimatic ranges, a higher number of subpopulations would be necessary in order to improve the knowledge of the effect of habitat quality in BMR.

Differences in metabolic rate are also relevant between sexes. The basic energetic threshold required by females was higher than males under both bioclimatic levels. Similar result was found by Boratyński and Koteja (2009) and Boratyński *et al*. (2010) with wild populations of *Clethrionomys glareolus*. Reproduction is one of the most costly processes in mammals (Gittleman and Thompson, 1988; Harvey, 1986), therefore a parental provisioning could be profitable to ensure the reproduction success. In this way, when females have an elevated minimum metabolism, this suggests that they have a higher energy potential to deal with the maternity and the breeding of offspring with more guaranties (Boratyński *et al*., 2010). However, an elevated BMR could be counterproductive in resource-poor habitats, so that the selected BMR must reach a compromise with the nutritional characteristics of the territory. Our study reveals differences in RMR values of females between bioclimatic ranges which may reflect an uneven availability of food resources along the year. Mueller and Diamond (2001) pointed out that animals from variable environments might have evolved to lower MR to ensure the metabolism in less productive periods. Hence, the environmental variability in mesomediterranean subpopulations could explain the lower RMR of these females. On the other hand, there are not significant differences in the RMR of males; the value of the supramediterranean-individuals is slightly higher than for the mesomediterranean. The energetic inversion in males do not require an additional level with which face breeding costs, so they have less RMR than females for the same habitat conditions.

According to Hayes (1989) and Willmer *et al*. (2005), BMR is intrinsically joined to the maximal aerobic metabolic rate or maximum rate of oxygen consumption. This latter rate represents the maximum physical activity or maximum thermoregulatory cost that an individual can support

(Weibel *et al*., 2004). In this sense, a priori, a high BMR could be advantageous because it could pose a high level of available energy to escape from depredators or survive under cold temperatures, however it also could imply disadvantageous effects as consequence of the food search to maintain that higher energetic level (*e.g.* increase in the exposition to depredators) (Brown, 1988; Lovegrove, 2000). The RMR of *M. cabrerae* is the compromise reached by the species between the nutritional quality of their habitats and their activity habits, maybe a higher RMR could favour Cabrera voles but their environments would not be able to keep it.

In short, the study of the metabolic behaviour from an intraspecific approach provides a more realistic knowledge of the response of the species to an environmental gradient in contrast to interspecific studies which approximate the species' responses with average trends. Our intraspecific approach allowed us identifies differences in metabolic rates with changes in habitat conditions, mainly associated to thermal environments. Thus, thermoregulatory mechanisms seems to be locally adapted to bioclimatic zones, so that under warmer conditions the subpopulations' strategy was a later beginning of TNZ and higher Cm, and vice versa. Therefore under a framework of the global change, detecting the physiological assembling of the species to the environment can provide new insides to design effective protection programmes to improve the conservation status of an endemic species as Cabrera voles.

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Capítulo 2

The intraspecific variability in the energetics of a habitat-specialist species: a common garden approach to study phenotypic plasticity and adaptation

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Manuscrito en preparación.

Abstract

The intraspecific study of physiological traits, such as basal metabolic rate (BMR), can provide valuable knowledge on the fit of a species to the environment, which can be used to improve predictive species distribution models used to study effects of global change. Identifying if physiological variability was adaptive or consequence of phenotypic plasticity of organisms is essential to delimiting tolerance thresholds and defining conservation strategies. We studied the Cabrera vole (*Microtus cabrerae*), a *Near Threatened* habitat specialist endemic to the Iberian Peninsula with a decreasing trend in its occupied areas. Cabrera voles were captured along an altitudinal range from mesomediterranean (more xeric) to supramediterranean (more mesic) bioclimatic zones and subjected to a common garden experiment to remove the effect of acclimatization and evaluate potential adaptive or plastic responses in their respective BMRs.

BMR differed between supra- and mesomediterranean voles, however the BMRs were not altered with the common garden period for any of the subpopulations. Thus, the different BMRs between bioclimatic zones could be attributed to an irreversible non-genetic physiological adjustment (*e.g.* maternal effects) or local adaptation. Although phenotypic plasticity was discarded, we could not identify the responsible mechanism because our breeding experiment was unsuccessful. Our results indicate that a specialist species such as *M. cabrerae* may be able to develop some physiological adaptation to cope with changes in the thermal environment. However, as the temporal scale and breadth of this capacity is unknown, we must be cautious when interpreting these outputs as indicators of a higher adaptive capacity to novel conditions.

Key words. BMR; Common garden; Ecophysiology; Global change; *Microtus cabrerae*.

Introduction

Global change is evident, causing shifts in species phenologies, organism traits, geographic ranges, and productivity as well as the disruption of diverse species interactions (Sala *et al*., 2000; Parmesan, 2006; Bozinovic & Pörtner, 2015). Thus, the maintenance and continuity of many ecosystems may be uncertain (Wang & Schimel, 2003; Bellard *et al*., 2012). The consequences of global change may have a dramatic effect on biodiversity since species' survival would depend on their capacity to adapt to novel conditions, leading to niche evolution (McGuire & Davis, 2013; Lawson & Weir, 2014). Alternatively, they could migrate in search of their ecological requirements or, ultimately, become extinct, if the species shows niche conservatism (Peterson *et al*., 1999; Wiens *et al*., 2010; Tkach *et al*., 2014).

Theoretical ecologists and environmental managers are developing different models and predictions of how biodiversity will be impacted by global change (*e.g.* Araujo *et al*. (2011)). They concur that these models have severe limitations because they lack a mechanistic basis. For example, the risks and vulnerabilities of individuals, species and ecosystems due to global change may be greatly under- or overestimated, depending on the relationship of the model to physiological requirements (Araujo *et al*., 2013; Bozinovic *et al*., 2014). To solve this problem, physiological traits can be correlated with a set of abiotic factors to infer the geographical distribution of the species and the spatial variation in their abundances (Bozinovic & Rosenmann, 1989; Bozinovic *et al*., 2011), and to disentangle how they influence the physiological performance of the species. Although the study of physiological characters has traditionally been addressed using an interspecific approach (Scholander *et al*., 1950; McNab, 1983; Bozinovic & Rosenmann, 1988; Lovegrove, 2003; Williams *et al*., 2004), intraspecific analysis can provide a deeper understanding of the specific mechanisms involved in each taxon, and then developing better predictions based on species-dependent responses to environmental changes (Hayes, 1989; Laugen *et al*., 2002; Novoa *et al*., 2005; Tieleman, 2007). Indeed, intraspecific differentiation can help us infer genotypic adaptation, because intraspecific differences can be explained by mechanisms linked to genotypic changes (Hayes & O'Connor, 1999; Bozinovic *et al*., 2009). Alternatively, these differences could be due to phenotypic plasticity through variability in the expression of the same genotype across environments with the consequent acclimatization to the habitat (Nespolo *et al*., 2001; Sassi & Novillo, 2015). Adaptive changes or phenotypic plasticity lead to increased individual fitness and similar phenotypic responses to environmental challenges (Schlichting & Pigliucci, 1998; Spicer & Gaston, 1999).

The study of intraspecific physiological variability is mainly addressed with common garden or transplant experiments (Spicer & Gaston, 1999). In a common garden design, a common environment with controlled conditions is imposed on the species (*e.g.* Tieleman (2007); Bozinovic *et al*. (2009)), whereas transplant experiments exchange individuals between populations subjecting them to their respective environments (*e.g.* Iraeta *et al*. (2006)). Both experimental designs exclude physiological differences attributable to local acclimatization thanks to the homogenization of the environment allowing the identification of other mechanisms, although genetic adaptation cannot be directly inferred from residual differences (Spicer & Gaston, 1999). Therefore, in order to distinguish a genetic mechanism from a non-genetic mechanism with permanent physiological effects, filial generations must be bred under the same conditions as the parental generation (*e.g.* Tracy and Walsberg (2001)). However, as the breeding and rearing of wild animals in the laboratory is not always feasible, the literature is usually restricted to studies that do not include wild animal reproduction in the laboratory. In any case, common garden experiments continue to be a powerful tool to examine the adaptive nature of physiological variability in the wild.

Among the available physiological parameters, basal metabolic rate (BMR) is one of the most used due to its standardized value in the energetic balance of organisms (McNab, 2002; Bozinovic *et al*., 2009), and is commonly considered an integrative measure of ecological and evolutionary physiology with a positive link to fitness (Cruz-Neto *et al*., 2003). BMR is reached within the thermoneutral zone (TNZ), *i.e.* the range of ambient temperatures where metabolic expenditure and heat production are minimized (Willmer *et al*., 2005). Thus, in order to evaluate the origin of the variability of physiological traits, we focused our study on the BMR of an endemic and endangered microtine rodent, the Cabrera vole, *Microtus cabrerae* in a common garden framework.

The Cabrera vole is an Iberian endemism, catalogued by the IUCN Red List of Threatened Species as Near Threatened on the international scale (Fernandes *et al*., 2008). It has a patchy distribution, restricted to a specific habitat of perennial grasses of *Agrostis castellana* and/or *Stipa gigantea* and rush beds in open areas with a high water table (Fernández-Salvador, 1998, 2007). The spatial distribution of the species is mainly circumscribed to the central strip of the Iberian Peninsula, with a slight extension towards the south-east and south-west (Garrido-García *et al*., 2013), over an altitudinal range from 250 m to 1500 m (Fernández-Salvador, 2007). Animals are arranged in family groups in territories of 300 m^2 on average (Fernández-Salvador, 1998), and are generally subjected to high levels of anthropogenic pressure (due to farming,

agriculture and infrastructure) (Fernandes *et al*., 2008). Thus, Cabrera vole subpopulations tend to be increasingly fragmented, and the number of occupied areas is declining (Fernández-Salvador, 1998; Pita *et al*., 2006; Pita *et al*., 2007; Fernandes *et al*., 2008; Garrido-García *et al*., 2013). Furthermore, the intrinsic habitat specialization of the species may endanger its persistence under a scenario of global change (Brouat *et al*., 2004). Therefore, it is highly important to identify the mechanisms behind the intraspecific physiological differences in this habitat specialist, since this knowledge can help clarify local adaptations allowing the persistence of the species and help to understand the loss of suitable territories given the deterioration of local conditions. Because the species inhabits a relatively wide altitudinal range with contrasting climatic conditions, it offers an opportunity to test whether BMR observed throughout these changing conditions is conserved when individuals are placed in a common environment. Previous research on Cabrera voles in the Central System of the Iberian Peninsula has shown energetic differences according to their origin. Indeed, subpopulations from the mesomediterranean bioclimatic zone have lower BMRs than their conspecifics from the supramediterranean bioclimatic zone (Castellanos-Frías *et al*., 2015). However, it is unknown whether the mechanism that controls intraspecific differentiation is due to acclimatization, explained by phenotypic plasticity, or genetic adaptation (*i.e.*, local adaptation). Hence, our goal is to determine the origin and magnitude of the differences in basal metabolic rate between subpopulations of *M. cabrerae*. Comparison of BMR values from different subpopulations in a common garden experiment can be a first step to understanding whether differences in physiological traits have a genetic basis or whether subpopulations show plastic responses to climate gradients.

Materials and methods

Experimental design

Field sites were defined according to bioclimatic zones. Different subpopulations distributed throughout the two bioclimatic zones were selected to obtain a variability of the sample of individuals within these zones. Thus, five subpopulations were used for the supramediterranean bioclimatic zone (subpopulations between 900 and 1100 m a.s.l) and six for the mesomediterranean zone (subpopulations between 480 and 580 m a.s.l) in Madrid, Spain (Fig 1). The two regions differ in climatic conditions and environmental variability in food and shelter resources. The supramediterranean zone is on average wetter and colder than the mesomediterranean zone (AEMET, 2015), and the configuration of the vegetation also differs

between the two: subpopulations at higher altitudes occupy wide areas, mainly with a dense canopy of *S. gigantea*, sometimes replaced by *A. castellana* and low densities of rushes (*Juncus* spp.), while the subpopulation in the mesomediterranean zone occupies markedly smaller areas with scattered patches of *A. castellana* and rushes (*Juncus* spp.).

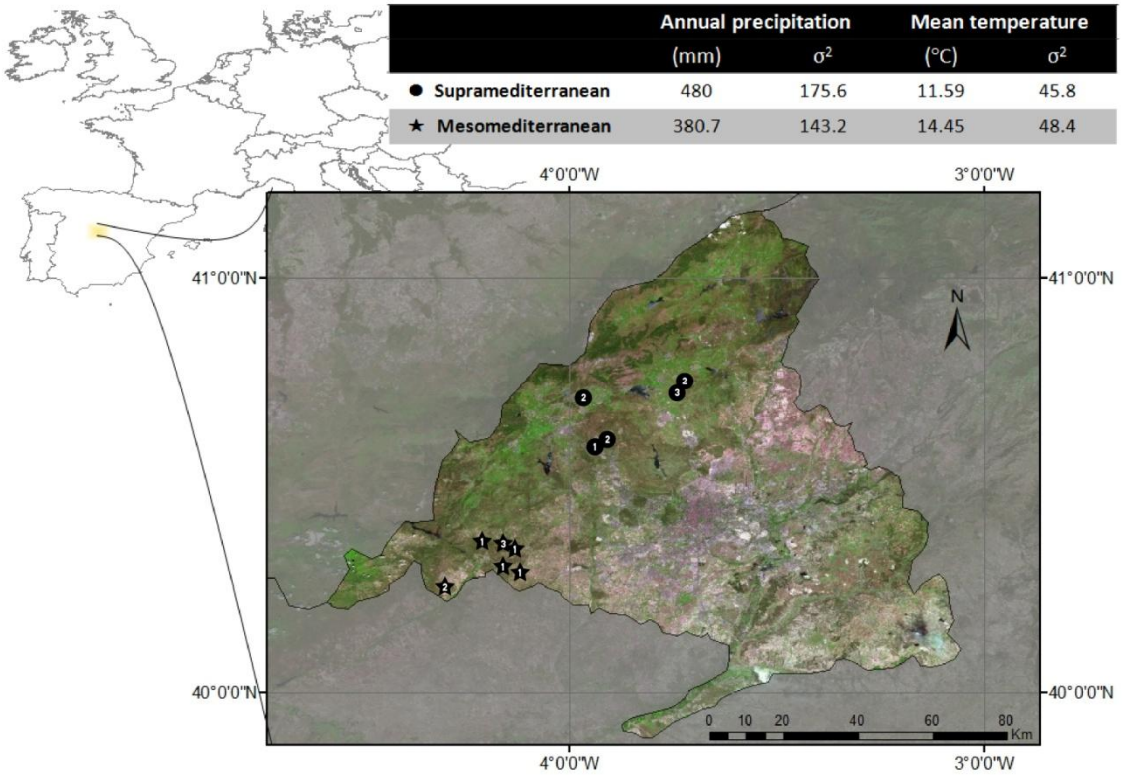

Figure 1. Geographical location of the studied Cabrera vole subpopulations. Mean annual precipitation, mean temperature and their respective variance are recorded for each bioclimatic zone. Numbers in the symbol are individuals captured in the respective subpopulation.

In the spring of 2014 and 2015, we captured nineteen *M. cabrerae* adults: 7 males and 3 females in the supramediterranean zone and 4 males and 5 females in the mesomediterranean zone. Pregnant females were not captured. Sherman live traps supplied with apples were placed in the runways that the species build in the vegetation. Trapping occurred during the day since the species presents diurnal and nocturnal activity (Fernández-Salvador, 1998). Due to its conservation status, we minimized the number of captures for this study. It was not possible to obtain balanced numbers in terms of gender because of the low capture success in this species.

Common garden experiment

Cabrera voles were kept in individual glass terrariums with double ventilation (60 x 30 x 30 cm) and a base of blonde peat substrate to maintain habitat moisture, hay and rocks to simulate their natural conditions. They were fed with apples and oat *ad libitum* (Castellanos-Frías *et al*., 2015). For 70 (± 10) days, animals were kept under a common environment with identical ambient temperatures (22° ± 3° C), moisture and a natural photoperiod, namely the *common garden period*. After this time, the metabolism of each animal was measured. All procedures were approved by the Animal Ethics Committee of the Rey Juan Carlos University of Madrid (Spain).

Metabolic measures

Following the methods outlined in Castellanos-Frías *et al*. (2015), the metabolism of the Cabrera voles was measured through the rate of oxygen consumption (VO_2) registered with a computerized open-flow respirometry system (Sable Systems, Anderson, NV). Briefly, animals were placed inside a glass metabolic chamber 7.4 cm in diameter (30.9 cm longitude) over a metallic grid that isolated the animals from their own excrement and allowed them to be in a comfortable position during measurements. A flow of 800 ml·min^{-1} of dried atmospheric air was pumped inside the metabolic chamber through a series of Drierite (H_2O-absorbent) and Soda Lime (CO_2-absorbent) filters placed at the inlet and outlet of the chamber to avoid the influence of H_2O and CO_2 on O_2 measures. The temperature inside the chamber was maintained and controlled with the PELT5 Temperature Controller (Sable Systems, Anderson, NV). Chamber output flow was analysed in the Foxbox Oxygen Analysis System (Sable Systems, Anderson, NV). ExpeData data acquisition software (http://www.sablesys.com) and equation 4a of Withers (1977) were used to express oxygen consumption as a metabolic rate.

Each animal was subjected to two metabolic measurements: one within 24 h after field capture and one after the common garden period. In both cases, the experimental protocol was identical. Cabrera voles were placed in the metabolic chamber and kept there for 45 min under TNZ conditions (temperature of 34º C) (Castellanos-Frías *et al*., 2015) to stabilize the respirometry instrumentation and ensure the relaxation of the animals during subsequent metabolic measurements. Measurements were then taken during the following 4.5 h under the same conditions, and basal metabolic rate was defined as the lowest stable consumption of

oxygen for at least 10 min. Metabolic rates were expressed as mass-specific metabolic rates (ml $O_2 \cdot g^{-1} \cdot h^{-1}$).

Body mass was registered before and after each metabolic measurement with an electronic scale (±0.1 g), and anal temperature at 5 mm deep (T_b) was recorded with a digital thermocouple probe (±0.1 °C) (OMEGA, model KMTSS) after each metabolic measurement. None of the animals died during the experiment.

Breeding experiment

To clarify the origin of the physiological differences between Cabrera voles from different bioclimatic zones, we designed a breeding experiment with the animals already acclimated to the common garden period. When we finished all metabolic measurements, males and females from the same bioclimatic zone were paired off and kept under the same laboratory conditions. We minimized the handling of the animals and entry into the room during this phase to avoid inducing stress. However, no effective breeding was achieved after 6 months of pairing. Consequently, the global aim of our study had to be less ambitious, and we restricted the analysis of physiological differences to changes produced within a single generation. At this point, all animals were released into their corresponding subpopulations.

Environmental variables

Environmental conditions surrounding the subpopulations were evaluated through climatic variables and a primary productivity index. Climatic information was obtained from current high-resolution data (30 arc-seconds) available from WorldClim (Hijmans *et al*., 2005). We selected the eleven climatic variables (S1 Table) that best explained the characteristics of the Cabrera voles' habitats. For the primary productivity index, we used the Normalized Difference Vegetation Index (NDVI) (Pettorelli *et al*., 2005) as a proxy of primary productivity. NDVI values were derived from MOD13Q1, a satellite image (spatial resolution: 250 x 250 m; temporal resolution: 16 days) from the MODIS sensor aboard NASA's TERRA satellite. One satellite image corresponding to the capture date of the voles was selected for each subpopulation. All information was incorporated into ArcGIS 10.1 to extract the climatic and NDVI values for each subpopulation. Dissimilarities in environmental variables between bioclimatic zones were evaluated through ANOVAs.

Statistical analysis

A linear mixed model (LMM) was used to evaluate the effect of the environment on the basal metabolic rate of Cabrera voles, applying the *lmer* function of the statistical package lme4 (Bates *et al*., 2014). LMMs are a good tool for analysing data with a structure of repeated measures and a mixture of fixed and random factors (Zuur *et al*., 2009). Through this analysis we evaluated the effect of the fixed factors: body mass, sex, bioclimatic zone of origin and the common garden period on BMR, controlling the variable contribution of each vole (random factor). Following the methodology provided by Zuur *et al*. (2009), a set of 19 models was evaluated with the corrected Akaike Information Criteria (AICc), which is more suitable than AIC for datasets with low sample sizes (Burnham & Anderson, 2002). The selection of models with AICc is based on the parsimony principle, that is it is selected the model that explains the most deviance with the minimum number of parameters (Zuur *et al*., 2009). The chosen model maximizes the goodness of fit to the data while minimizing the complexity of the model, and the difference between the best model and second best model is greater than 2 units. So, when the difference in AICc between models was less than 2 units, it was not corrected to discard the others. The model average function (R function *model.avg*, library MuMIn; Barton, 2015) was used to select the best model with averaged parameters. All statistical analyses were developed in R software (R Core Team, 2018).

Results

Although Cabrera vole habitats have a well-defined structure and vegetal composition, differences in primary productivity were found between the studied bioclimatic zones (ANOVA, $F_{1,9}$=5.389, p=0.045). The analysis of climatic variables also revealed thermal differences between the two areas (see statistical parameters in the Supplementary Table S1).

The LMM revealed that the BMRs from post-field capture and post-common garden conditions were similar (Supramediterranean zone: 1.963 ± 0.498 and 1.941 ± 0.501, respectively and in the Mesomediterranean zone: 1.528 ± 0.56 and 1.589 ± 0.387, respectively).Therefore, the model used to explain BMR did not consider metabolic differences between the two measures. In the same vein, the LMM models did not include body mass factor as relevant to explain the BMR. As the best model identified differences in BMR according to the origin of the studied voles, bioclimatic zone was included as a fixed factor and individual as a random factor (Table 1). The best model parameters were averaged from the two best models with an AICc less than 2 units

(Table 1). The model explained 33% of the variability of BMR (conditional R^2 = 0.332), while the fixed factor of the model explained 15% of the variability (marginal R^2 = 0.146).

Table 1. Two best models with an AICc of less than 2 units. The best model is in bold and model coefficients are only given for the best model.

Model	Factor						logLik	D	ΔAICc	*wi*
	Fixed			Random						
1	**BZ**			**IND**			**-24.59**	**49.18**	**0.00**	**0.63**
p.w.	***1.00***									
		Intercept	BZs		IND	Residual				
	coefficient	1.506	0.538	variance	0.050	0.181				
	SE	0.145	0.269	SD	0.225	0.425				
2	BZ	S	BZ:S	IND			-22.37	44.75	1.07	0.37
p.w.	*1.00*	*0.37*	*0.37*							

For each model the fixed components (BZ: Bioclimatic Zone factor; S: Sex factor; BZ:S: the interaction between the two factors), random component (IND is each individual), logLik, D (deviance), ΔAICc (delta AICc is the difference in AICc of each model compared to the best model) and *wi* (model weight according to the model average) are described. Parameter weight (*p.w.*) describes the relative importance of the variable depending on the number of models in which the factor is included. The intercept of the best model summarizes the level of the mesomediterranean bioclimatic zone, while the supramediterranean level is indicated in BZs.

Under both field and laboratory conditions, the BMR of Cabrera voles from supramediterranean subpopulations was on average 0.538 ml O_2/g·h higher (35.7%) than that of mesomediterranean individuals (Table 1). However, only subtle differences were detected in the BMR of voles from each bioclimatic zone (Fig 2).

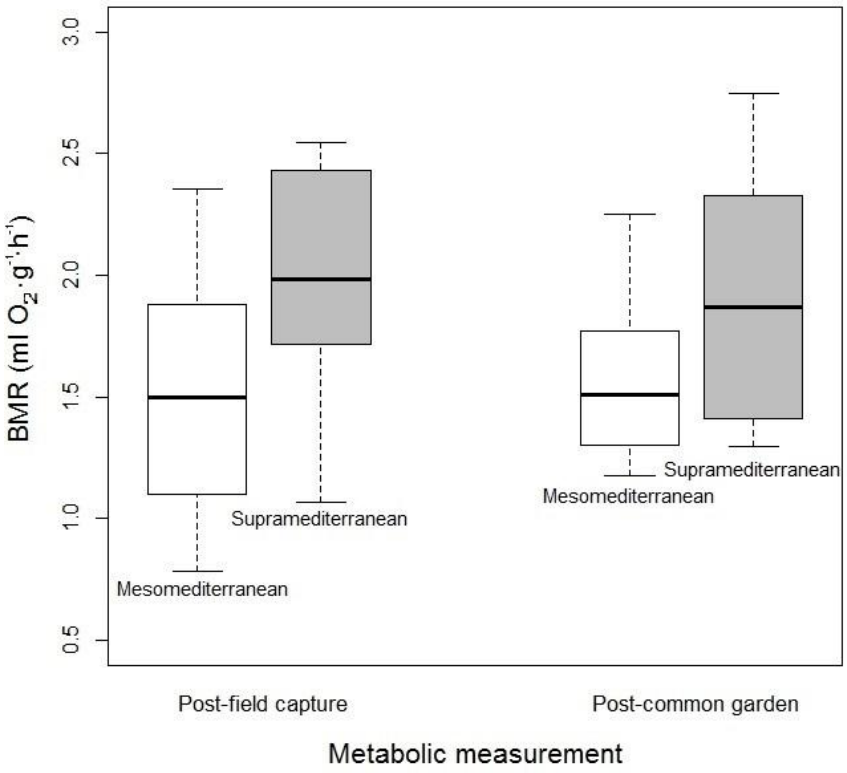

Figure 2. BMR of supra- and mesomediterranean subpopulations of *M. cabrerae* under post-field capture and post-common garden measurement conditions.

The rate of change in BMR in supramediterranean subpopulations was -0.022 ml $O_2 \cdot g^{-1} \cdot h^{-1}$ under the new thermal scenario, while individuals from the mesomediterranean zone showed a slight increase of 0.061 ml $O_2 \cdot g^{-1} \cdot h^{-1}$ under the same conditions.

Discussion

In addition to genetics, the environment also plays a role in determining the physiological state of animals. Thus, differences in environmental conditions due to the geographical range generally lead to differences in physiological traits at the interpopulation level (Spicer & Gaston, 1999). Intraspecific physiological variability associated with habitat conditions has been widely addressed in the literature for many taxa, such as isopods (Bozinovic *et al*., 2014), birds (Broggi *et al*., 2005; Cavieres & Sabat, 2008), small mammals (Bozinovic *et al*., 2009) as well as the studied species (Castellanos-Frías *et al*., 2015). In agreement with Castellanos-Frías *et al*. (2015),

our results indicate intraspecific variability in the BMR level of *M. cabrerae* as a consequence of environmental changes associated to the altitudinal range of its distribution. Both in the post-field capture and the post-common garden measurement, the BMR of supramediterranean individuals was higher than that of their conspecifics from lower altitudes. The two bioclimatic zones differ in biotic and abiotic conditions: they have different levels of primary productivity and different climatic conditions, which could promote the corresponding physiological differences between subpopulations.

Nevertheless, only slight variations were detected in metabolic rates compared to previous studies on the species (Mathias *et al*., 2003; Castellanos-Frías *et al*., 2015). The BMR obtained for supra- and mesomediterranean subpopulations were 23% and 26%, respectively, lower than the resting metabolism reported by Castellanos-Frías *et al*. (2015) and somewhat higher than the BMR in southern-Portuguese subpopulations (Mathias *et al*., 2003). Resting and basal metabolism are measured under similar experimental conditions, except that the former is not developed under a post-absorptive state (Wiens & Graham, 2005), which may explain these subtle metabolic differences. The study on southern-Portuguese Cabrera voles was conducted in subpopulations located between meso- and thermomediterranean bioclimatic zones (between 200 and 300 m a.s.l.) where, according to our analyses, they should have a lower BMR than the BMRs obtained in our study. In fact, Mathias *et al*. (2003) reported BMR values of 1.13 ml O_2/g·h, which is 45% and 25% lower than our supra- and mesomediterranean subpopulations, respectively.

Physiological traits such as BMR are widely reported as plastic characters (McDevitt & Speakman, 1994; Nespolo & Rosenmann, 1997; Cavieres & Sabat, 2008; Nuñez-Villegas *et al*., 2014), however our results do not support physiological flexibility in Cabrera voles. Although the animals (species with a longevity of one year (Feliu *et al*., 1997)) were kept under common garden conditions for almost two months, they maintained their basal metabolic rates without changes. Hence, they did not acclimate to the new thermal environment. Furthermore, the subtle change in BMR between metabolic measurements indicated a low rate of change and, thus, low phenotypic plasticity (Nespolo, 2000). O'Connor (1995) also found a low plastic capacity in house finches (*Carpodacus mexicanus*), whose metabolic response to the thermal environment did not differ throughout the year.

Although the thermal environment was homogenised with the common garden period, metabolic differences persisted between the two bioclimatic zones. The mass-specific BMR of animals from the supramediterranean zone was also higher than that of mesomediterranean

voles after the period under laboratory conditions. Consequently, the observed intraspecific differentiation in the Cabrera vole cannot be explained solely by acclimatization to their respective thermal environments. This pattern has also been identified in other species dwelling in contrasting habitats, such as the rodent *Abrothrix olivaceus* (Novoa *et al*., 2005) and the sparrow *Zonotrichia capensis* (Cavieres & Sabat, 2008). These two studies analysed the effect of climate on the metabolic rate of the species with animals acclimated to different thermal environments. Novoa *et al*. (2005) and Cavieres and Sabat (2008) also found that animals from colder and rainy areas had higher BMRs, even after acclimation treatments. These authors suggested that local differentiation could be explained by other mechanisms such as local genetic adaptation, developmental plasticity, reversible environment changes or a combination of these.

The persistence of intraspecific physiological differences even under controlled laboratory conditions could be due to an irreversible non-genetic physiological adjustment or genetic modifications such as local genetic adaptation. Some authors consider that physiological heterochrony could be an irreversible phenomenon (Spicer & Gaston, 1999), which can provide inter-population variability through a change in the relative timing of expression of phenotypic traits in one life stage (Schlichting & Pigliucci, 1998; West-Eberhard, 2003). Maternal effects are another non-genetic mechanism that can act as a cross-generational bridge of phenotypes, in which case the effect of parental phenotype can be extended throughout the offspring's life (West-Eberhard, 2003). On the other hand, the adaptation of subpopulations to their corresponding bioclimatic zone could imply changes in genotype to improve individual fitness. Unfortunately, assessing the mechanisms that determine the differences in the BMR of Cabrera voles required the breeding of a filial generation from both subpopulations. If metabolic differences had been removed in the filial generations, the mechanism would have been non-genetic, and *vice versa* (Spicer & Gaston, 1999). However, we were unable to evaluate this phenomenon since the wild Cabrera voles did not breed under laboratory conditions. In any case, we can discard phenotypic plasticity as a mechanism to cope with environmental changes in this species.

The Cabrera vole is a habitat specialist which requires habitats with perennial grass coverage throughout the year for feeding and shelter. However, the restrictive moisture and temperature conditions of the summer in the Mediterranean region reduces grass coverage and hence the size of the vole habitat, with the subsequent decrease in their carrying capacity. Although this restrictive season has an analogous effect in the two areas, it is especially notorious in the

mesomediterranean subpopulations since the reduction in rainfalls is greater (Peinado & Rivas-Martínez, 1987). Hence, these restrictive conditions could favour the fixation of physiological traits such as BMR (Spicer & Gaston, 1999) in both bioclimatic ranges, moulding a lower BMR in mesomediterranean subpopulations as a consequence of lower primary productivity, and a higher BMR in supramediterranean areas to face the higher thermogenic demand (see also (Castellanos-Frías *et al*., 2015)). In this vein, the food habits hypothesis addresses the variability in the BMR in relation to the food quality, food availability and food predictability (Cruz-Neto & Bozinovic, 2004; Bozinovic *et al*., 2009); thus low-quality or unpredictable diets are linked to low BMRs and vice versa (Bozinovic *et al*., 2009). The energetics of Cabrera voles bring to light the role of primary productivity on the variability of the BMR and agree the food habits hypothesis.

Our results contradict the "generalist-to-specialist" (Futuyma & Moreno, 1988) and the "specialist as a dead-end" (Moran, 1988) hypotheses. According to these hypotheses, evolution in generalist and specialist species is a one-way transition from generalist to specialist where the latter would lack adaptation capacity (Futuyma, 2001). On the contrary, our research reveals that a specialist species such as *M. cabrerae* may be able to develop adaptive changes in physiological traits to cope with changes in the thermal environment. Other studies have also identified adaptive diversification in habitat specialists in response to intrinsic habitat variability (Berven, 1982; Bouton *et al*., 1999; Brouat *et al*., 2004). This local adaptation at the interpopulation level is achieved when low dispersal movements are coupled with strong selective pressures (Kisdi, 2002; Kawecki & Ebert, 2004) until equilibrium is reached. However, as the adaptive ability of the species could be limited at the edges of high selection gradients (Kawecki & Ebert, 2004), the opportunities of Cabrera voles might be compromised under a harsh scenario like global change.

In this sense, the suitable area for this species may be highly reduced due to climate change, in agreement with the predictions of Araujo *et al*. (2011) who projected a 67% to 72% decrease in suitable habitat for the period 2041-2070. Only 2-4% of this territory coincides with that currently used by the species. Even though the species may develop some physiological adaptive capacity to withstand a tolerable threshold of climate change in its habitats, the temporal scale for developing this capacity is unknown. Thus, the conservation of Cabrera voles would most likely depend on dispersive phenomena on a large scale. In short, we must be cautious in interpreting that observed differences are the result of local genetic adaptation, and further studies should be carried out to clarify the adaptation capacity of this species and its breadth.

Supplementary Information captions

Supplementary Table S1. Statistical parameters of ANOVAs analyses between bioclimatic zones in climatic variables.

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Supplementary information

Supplementary Table S1. Statistical parameters of ANOVAs analyses between bioclimatic zones in climatic variables.

Variables	*F* value	*p* value
Annual precipitation	$F_{1,9}$ = 289.34	3.768e-08
Annual maximum temperature	$F_{1,9}$ = 410.16	8.128e-09
Annual mean temperature	$F_{1,9}$ = 411.43	8.019e-09
Annual minimum temperature	$F_{1,9}$ = 497.57	3.461e-09
Temperature seasonality	$F_{1,9}$ = 166.13	4.179e-07
Maximum temperature of warmest month	$F_{1,9}$ = 400.86	8.994e-09
Minimum temperature of coldest month	$F_{1,9}$ = 470.36	4.439e-09
Mean temperature of driest quarter	$F_{1,9}$ = 556.88	2.101e-09
Precipitation seasonality	$F_{1,9}$ = 152.82	5.975e-07
Precipitation of wettest quarter	$F_{1,9}$ = 207.17	1.614e-07
Precipitation of driest quarter	$F_{1,9}$ = 291.49	3.648e-08

Capítulo 3

Assessment of the effect of climate changes in the Late Pleistocene and Holocene on niche conservatism of an arvicolid specialist

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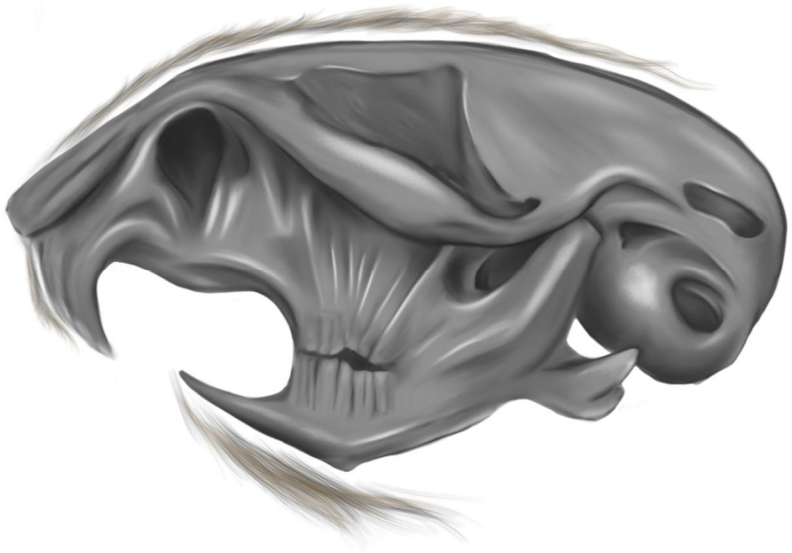

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Abstract

Climate change is not only evident, but its implications on biodiversity are already patent. The scientific community has delved into the limitations and capabilities of species to face changes in climatic conditions through experimental studies and, primarily, Species Distribution Models (SDMs). Nevertheless, the widespread use of SDMs comes with some intrinsic assumptions, such as niche conservatism, which are not always true. Alternatively, the fossil record can provide additional data to solve the uncertainties of species' responses to climate change based on their history.

Using a combined environmental (niche overlap indices) and geographical approach (temporal transferability of SDMs), we assessed the niche conservatism of *Microtus cabrerae* throughout its evolutionary history: the Late Pleistocene and the Holocene. The set of analyses performed within this timeframe provides a broad view pointing to a shift in the realized climatic niche of the species. Specifically, *M. cabrerae* exhibited a broader niche during glacial times than interglacial times, expanding towards novel conditions. Hence, the species might have developed an adaptive ability, as a consequence of mechanisms of local adaptation or natural pressures, or just have phenotypic plasticity to cope with the novel environment, due to expansion into an unfilled portion of the niche. Nevertheless, the more restricted realized niche during last interglacial times reveals that the species could be close to its physiological limits.

Key words. Cabrera vole; Ecological niche; Equivalency test; Niche overlap; Niche shift; Niche stability; Similarity test; Species distribution model; Temporal transferability.

Introduction

Global climate change is evident and its patent effects on biodiversity include changes in range boundaries, species abundances and phenological shifts (Parmesan, 2006; Parmesan and Yohe, 2003). Nevertheless, other effects might be important, since genetic diversity might also be affected both at the organism and biome level (animal communities) (Bellard *et al*., 2012; Yannic *et al*., 2014). Consequently, a wide array of experimental studies has tried to elucidate the limitations and capabilities of the species to identify their physiological thresholds (*e.g.* Hovenden *et al*. (2008); Neuwald and Valenzuela (2011)).

Species Distribution Models (SDMs) have been used to associate species occurrence with environmental predictors through mathematical algorithms to estimate the species' climatic niche (Elith *et al*., 2006). Although SDMs are widely used, some basic assumptions such as the ecological niche conservatism ("niche stability" *sensu* Nogues-Bravo (2009)) have been scarcely tested. Niche conservatism refers to the preservation of the ecological traits linked to the species' niche (Wiens *et al*., 2010). The conservation of the ecological niche of species in space and time remains controversial (Broennimann *et al*., 2007; Graham *et al*., 2004; Lawson and Weir, 2014; Peterson *et al*., 1999). The mechanisms of local adaptation to marginal conditions or natural pressures could induce evolutionary processes in the long-term (Alexander and Edwards, 2010; Sax *et al*., 2007), or just expansion into a previously unfilled portion of the niche (Veloz *et al*., 2012), therefore, some degree of variation in the species' niche might be expected (Holt and Gaines, 1992). However, whenever SDMs are aimed to forecast future climate change impacts, the niche conservation assumption implies a static snapshot of the species' environmental niche that ultimately underestimates the real abilities of organisms to cope with new environmental conditions. Specifically, SDMs do not consider the species' phenotypic plasticity to non-analogue climates, so their accuracy is reduced (Veloz *et al*., 2012). Moreover, a good performance of the models requires the stability of the biotic interactions and the dispersion ability shaping the realized niches of the species (Maiorano *et al*., 2013; Veloz *et al*., 2012). Some of these drawbacks can be palliated using other methodological approaches, such as community-level models (CLMs) (Maguire *et al*., 2016) or multi-temporal models to approximate the fundamental niche (Maiorano *et al*., 2013). Alternatively, the use of fossil records can assist to study the responses of the species to climate changes. Fossils result a useful and rich source of information about the grade of evolution within lineages or species and thus, conservation or shifts in niches. In fact, the SDM approach and paleobiological data can be combined as a tool to assess current distribution patterns and define conservation guidelines (Svenning *et al*., 2011). The most recent

part of the Quaternary (Late Pleistocene and Holocene) was characterized by periods of cold and warm climate conditions, such as the Last Interglacial Period (LIP), the Last Glacial Maximum (LGM) and the Mid-Holocene. The projection of current distributions models to those analogous, warmer or glacial climates can reveal suitable territories for the species under these scenarios (Fløjgaard *et al*., 2009). The subsequent evaluation of these models with an independent data set of fossil record may clarify the survival strategies and biological thresholds of the species (*e.g.* McGuire and Davis (2013); Worth *et al*. (2014)).

Interpretation problems can arise when SDMs are projected under other scenarios, since the geographical space is not likely to keep the same environmental range of values along the time periods considered. Hence, it is important to discern the niche-biotope duality to discern between the environmental and the geographical space occupied by the species (Guisan *et al*., 2014). The multidimensional environmental space can be summarized using techniques such as multivariate statistics to identify changes in niche features in space or time (*e.g.* Broennimann *et al*. (2012)). Therefore, a combined niche-biotope approach can provide complementary data to assess niche dynamics (Guisan *et al*., 2014).

The sensitivity of small mammals to local environmental changes makes them good indicators of climate conditions (García-Alix *et al*., 2008). Furthermore, their short life cycles make them suitable for the study of responses to either subtle or pronounced environmental changes. Small mammals reflect accurately any variation in niche conditions (Bilton *et al*., 1998). In this study, we assess a possible shift in the climatic niche of the Cabrera vole (*Microtus cabrerae*), an endemic arvicoline of the Iberian Peninsula, accounting for its evolutionary history. *M. cabrerae* has a reduced distribution range compared to its fossil record (Laplana and Sevilla, 2013), which included locations in south-eastern France and north-eastern Spain. However, the species distribution is currently limited to a fragmented area in central-south Portugal and Spain (Fernandes *et al*., 2008). Agriculture combined with urban infrastructures have been identified as the main causes of the current population decline (Fernández-Salvador, 1998, 2007; Holt and Gaines, 1992). Nevertheless, the effect of climate on the niche of the Cabrera vole' seems to play an important role (Castellanos-Frías *et al*., 2015). The species only inhabits in perennial grasses of *Agrostis castellana* and/or *Stipa gigantea* and rush beds in open areas with a high water table (Fernández-Salvador, 1998, 2007). Therefore, the species could be expected to expand its favourable habitats under humid conditions, while xeric conditions could decrease habitat suitability. Araujo *et al*. (2011) and Mestre *et al*. (2017) predict the species to experience a northward range shift and, a strong contraction of the southern portion of its distribution. As

this habitat-specialist species has persisted despite adverse climate changes, *M. cabrerae* may have some adaptive ability to face the restrictions. We hypothesized that cold conditions (*e.g.* during the Last Glacial) might not be as limiting to the species as a warm, arid scenario (*e.g.* during the Last Interglacial). Therefore, we aim to elucidate on the Cabrera vole's ability to cope with climate change since the beginning of its history and to clarify whether climate alone explains the species' current distribution. Using an environmental and geographical approach we assessed the niche dynamics of *M. cabrerae* that have occurred during the climate changes since the Late Pleistocene. Specifically, we projected a current SDM to three paleoclimate scenarios (and vice versa) and measured the climatic overlap of current and paleo-niches to evaluate the niche conservatism of the species.

Materials and methods

Environmental variables: current and past scenarios

The environmental framework was defined through a set of nineteen bioclimatic variables from WorldClim with 2.5 min (approx. 4.6 km x 4.6 km) spatial resolution (Hijmans *et al*., 2005). To minimise multicolinearity in predictors, the variables were first arranged in a cluster dendrogram with Pearson correlations. One bioclimatic variable was selected for each branch (Pearson correlation: $r < \pm 0.7$) based on the biology of the species and the ability of the model to distinguish the regions of the environment and vole occurrences. The subset of variables was also filtered with a variance inflation factor: VIF<5 (Venier *et al*., 2004) (R library HH; Heiberger, 2017) to remove the predictors already explained by the rest of the variables. Finally, the explanatory variables were reduced according to the variable importance analyses from the conducted SDMs (Broennimann *et al*., 2012). In the end, a set of five bioclimatic variables (Supplementary Fig. S4) was applied in all SDMs, the principal components analysis (PCAs) as well as subsequent analyses.

The selected bioclimatic variables reflect the biological restrictions of the current distribution of *M. cabrerae*. The species is a specialist of grassland habitats with marked Mediterranean conditions (Fernández-Salvador, 1998) and avoids the Euro-Siberian zone (Pita *et al*., 2014), characteristics represented by the temperature seasonality variable (Bio 4). The other variables delimit suitable areas for the species in temperature in the wettest and driest quarters (Bio 8 and Bio 9), and precipitation in the warmest and coldest quarters (Bio 18 and Bio 19).

Temporal transferability was evaluated for 4 climate scenarios: current climatic conditions and 3 paleoclimatic scenarios corresponding to the Last Interglacial Period dated around 128-116 ka BP (the Marine Isotope Stage 5e or MIS5e) (Kukla *et al*., 2002), the Last Glacial Maximum, 21-19 ka BP ago (inside the MIS2: 28-11.7 ka BP) (Jost *et al*., 2005; Lorius *et al*., 1985; Müller *et al*., 2011) and the Mid-Holocene, 6 ka BP ago (inside the MIS1: 11.7 ka BP to present time) (Davis *et al*., 2003; Lorius *et al*., 1985; Müller *et al*., 2011) (see the average values of bioclimatic variables in Supplementary Fig. S4). The LIP was a warm period at the beginning of the Late Pleistocene. The climate during LIP was highly continental with marked seasonality changes, and it was generally slightly dryer than the present with increasing precipitation from inland to marine areas (Brewer *et al*., 2008). In contrast, conditions towards the end of the Late Pleistocene were notoriously cold and arid due to the Last Glacial Maximum during MIS2. In spite of the harsh climate in Europe (Jost *et al*., 2005) LGM in the Iberian Peninsula presented cold temperatures, but aridity levels were not very low. In fact, there were periods with relatively high water availability in the Mediterranean region (Morellon *et al*., 2009). The glacial landscapes were dominated by cold steppe formations with coniferous forest patches and mesophytes and thermophytes refuge areas (Sánchez-Goñi *et al*., 2002). The Mid-Holocene brought an improvement for the species in climate conditions, which were once again warmer and moister (Morellon *et al*., 2009). As a result, the Mediterranean region was covered by deciduous forests (Magny *et al*., 2002).

Paleoclimate data were derived from the simulations of the Community Climate System Model (CCSM) global circulation model (GCM). CCSM was used for LIP data, while Mid-Holocene and LGM were developed with CCSM4 (Hijmans *et al*., 2005).

Occurrence and fossil record datasets

We obtained a total of 404 current occurrence records from the Spanish inventory of terrestrial species (Ministry of Agriculture Food and Environment, 2016), Mira *et al*. (2008) and Garrido-García *et al*. (2013) to establish the global distribution of *M. cabrerae*.

Fossil records were obtained from the species' review by Laplana and Sevilla (2013) and completed with the records in Cuenca-Bescos *et al*. (2014). Since fossil records were classified in wide periods, we only considered records with a chronology within the paleoclimate scenarios, spanning the whole period (Supplementary Table S1). A total of 28, 16 and 13 fossil records were used for Mid-Holocene, LGM and LIP, respectively.

To avoid statistical artefacts related to spatial autocorrelation, only one datum of the species was considered for each 10-km cell of the territorial grid, both in occurrence and fossil records.

Niche modelling

Niche conservatism of *M. cabrerae* was evaluated using a combined methodological approach: a geographical analysis using SDMs and environmental assessment with several statistical tests of niche overlap. We compared geographical projections and environmental analyses of the current environment with the corresponding geographical projections and environmental analyses for each period in paleoclimate scenarios over the native territory. Thus, the analyses of the current distribution of Cabrera voles comprised environmental conditions of the Iberian Peninsula, while the modelling of the paleo-scenarios considered the environment of the Iberian Peninsula and the French region. All statistical analyses were carried out in R (R Core Team, 2018).

Geographical analysis: Species Distribution Models

The geographical approach was based on the temporal transferability of SDMs. The SDMs were built with three robust modelling algorithms: Generalized linear model (GLM), Random Forest (RF) (R library randomForest; Liaw and Wiener, 2002) and Maximum Entropy (Maxent; Phillips *et al*., 2006) (R library dismo; Hijmans *et al*., 2016). We specifically included GLM because of its flexibility to control all the factors involved in the model such as interactions or variable fitting, while Maxent and RF were used for their good predictive performance (Elith *et al*., 2006; Gonzalez-Irusta *et al*., 2015). All models were built using current occurrence data of the species plus a background dataset (with 20% of the points in the global territory considered) for Maxent and weighted background for the GLM algorithm, whereas the RF and GLM were developed with a set of pseudoabsences. To achieve the best fit between the ecological response curves of the species and the modelled ones, we redefined the structure of the models, *e.g.* the GLM's degree of the polynomials of the environmental variables or the features of Maxent. We also tried to minimize the complexity of the models. Thus, we conducted several Maxent models with different regularization parameters to finally fix this parameter at 3 units based on the criteria of the adjustment to ecological curves and gain values. Higher values of the regularization parameter prevent over-fitting model predictions to the presence locations values (Merow *et al*., 2013). Model fit was assessed by explained variance for RF and GLM (called deviance or D^2) and

by gain for Maxent (closed to deviance of GLM), as both measure model quality (Merow *et al*., 2013; Yost *et al*., 2008).

Finally, all models were evaluated by a bootstrapping validation procedure consisting of 20 times iterative data partitioning (40%-60%, testing-training, respectively), using the area-under-the-curve (AUC) test. The AUC is a threshold-independent measurement that assesses the true positive fraction versus the false positive fraction of SDM predictions (Fielding and Bell, 1997), providing a single model performance score (Phillips *et al*., 2006).

Models were projected to the three paleoclimatic scenarios to predict Cabrera vole paleo-distribution, and thus identify a possible change in the climatic niche of the species. Niche predictions were assessed by the AUC test and the fossil record, an independent dataset of presence.

Similarly, all algorithms were calibrated for each of the paleoclimatic scenarios, following the previously described methodology, and transferred to the current climate scenario. The niche projections for the current climate framework were evaluated with the AUC test using the 404 points of current species occurrence.

The most dissimilar variable analysis (MoD) of Maxent 3.3.3k software (Phillips *et al*., 2006) was conducted for projected variables from current to paleoclimatic scenarios. This test reveals the variables and regions where a projected variable falls outside the calibrated range of values.

Environmental analysis: PCA analysis

From an environmental perspective, we evaluated the degree of similarity or differentiation in the ecological niches throughout the species' history.

First, the global climatic space from all scenarios (current and the three paleo-scenarios) were summarized in the two-dimensional space of the PCA (R library ade4; Dray and Dufour, 2007). In this framework, individual hyperspaces were created to represent the current and one of each paleo-niches of the species (repeated for the three paleo-scenarios). The environmental hyperspace of the occupied areas of the different scenarios was compared to analyze changes in the species niche. Niche overlap was assessed based on the Schoener's D index, an ecological space-based metric which ranges between 0 (no niche overlap) and 1 (both niches have identical environmental spaces) (Warren *et al*., 2008).

Following the methodology proposed by Warren *et al*. (2008) and Broennimann *et al*. (2012), we calculated two simulation-based tests to identify overlap patterns of current and past niches of

Cabrera voles: the niche equivalency and the niche similarity tests (R library ecospat; Broennimann *et al*., 2015). The niche equivalency test determines whether two niches are identical when their occurrences are reallocated. Species occurrences in the two temporal scenarios (the current and one of the paleoclimatic scenarios, successively) were pooled and randomly split 100 times into two datasets with the same number of data as in the original scenario to conduct new niche models and their respective D metrics. This random process allowed us to create a null distribution of D values for comparison with observed D. So, when the D value was inside the 95% confidence interval of the null distribution, we could assume that the niches were equivalent. The niche similarity test focuses on both the environment of the occurrences and the environment of the background dataset. Therefore, this test analyzes if niches are more similar or different from one another than expected by local similarities. In this case, one of the niches was created from randomly gathered occurrences in the available environment and compared to the other niche to determine the D metric of overlap. The process was iterated 100 times to obtain the null distribution of D values. So, when measured D was outside 95% of the simulated distribution, the species occupied areas more similar or different than expected by chance under both scenarios. As the niche similarity test was assessed in both directions (*i.e.* from the current climate to a paleoclimate scenario and vice versa).

Additionally, the niche dynamics of *M. cabrerae* in the different scenarios were evaluated with the indices of expansion, stability and unfilling (R library ecospat; Broennimann *et al*., 2015). Niche expansion referred to conditions inside the paleo-niches that were not included in the current niche. On the contrary, unfilling represented conditions from the current distribution, which were not available in the paleo-niche. The stability index denoted common conditions throughout the niches of the Cabrera voles (Guisan *et al*., 2014).

Results

Climatic niche using a geographical approach

Among the modelling algorithms, the RF model had the best performance in predicting current species occurrences (Table 1). This algorithm explained the 53% of the variance despite only considering climatic variables. Although all models had a good current predictive ability, none accurately located the fossil records of Cabrera voles when projected into paleoclimate scenarios. Model validation with the fossil record (independent data) provided poor AUC values

(range: 0.302-0.670), indicating that the models did not perform better than a random prediction.

Table 1. Modelling algorithms used to identify the paleo-niches of *M. cabrerae* from the current distribution. Models are calibrated under current conditions and projected to current and paleoclimate scenarios. Model performance is evaluated through a double approach: the statistical assessment of the model with the variance explained and the predictive ability of each model in current (internal validation) and past scenarios (external validation) with AUC.

Model	Dataset	Explained variance		AUC			
		Variance	Gain	Current	Mid-Holocene	LGM	LIP
GLM	pseudo	0.373		0.878	0.662	0.613	0.402
RF		0.530		0.967	0.670	0.414	0.302
Maxent	bg		0.594	0.836	0.634	0.598	0.462
GLM		0.325		0.849	0.653	0.625	0.408

pseudo: pseudoabsences; bg: background

Both GLM deviance (R function *D-squared*, library modEva; Barbosa *et al*., 2016) and RF variance (R library randomForest; Liaw and Wiener, 2002) are expressed as a decimal. Whereas, gain value from Maxent defines the exponent to generate the average likelihood of presences, therefore the maximization of gain results in a model that best discriminates presence and background data (Merow *et al*., 2013; Yost *et al*., 2008). The AUC of the current scenario was obtained from a bootstrapping evaluation; on the other hand, the AUCs from past transferabilities were calculated using fossil records as independent occurrence points.

All models were accurate in predicting the location of suitable territory currently used by the species. Only geographical projections of the best algorithm (RF) are summarized in Fig. 1, although the remaining models followed a similar trend. Temperature seasonality was the most explanatory variable in the models, since its random permutation had the greatest effect in the model predictions (Fig. 2); followed by precipitation variables. The species showed a preference for areas with a marked Mediterranean climate, as Cabrera voles occurrences were concentrated in areas with high temperature seasonality values (Supplementary Fig. S1) up to a threshold (temperature seasonality values above *ca.* 6700 units) at which occurrence was again minimized. Consequently, the current niche predicted for the species was the central, south-western and south-eastern strips of the Iberian Peninsula (Fig. 1a). The most northern populations (Fig. 1a) were not accurately identified with any of the current SDMs, since the values of the bioclimatic variables in this area were outside the range commonly preferred by the species (Supplementary Fig. S1). Some degree of spatial transferability of Cabrera voles beyond their current distribution limits was found in south-eastern France, as shown in Supplementary Fig. S2.

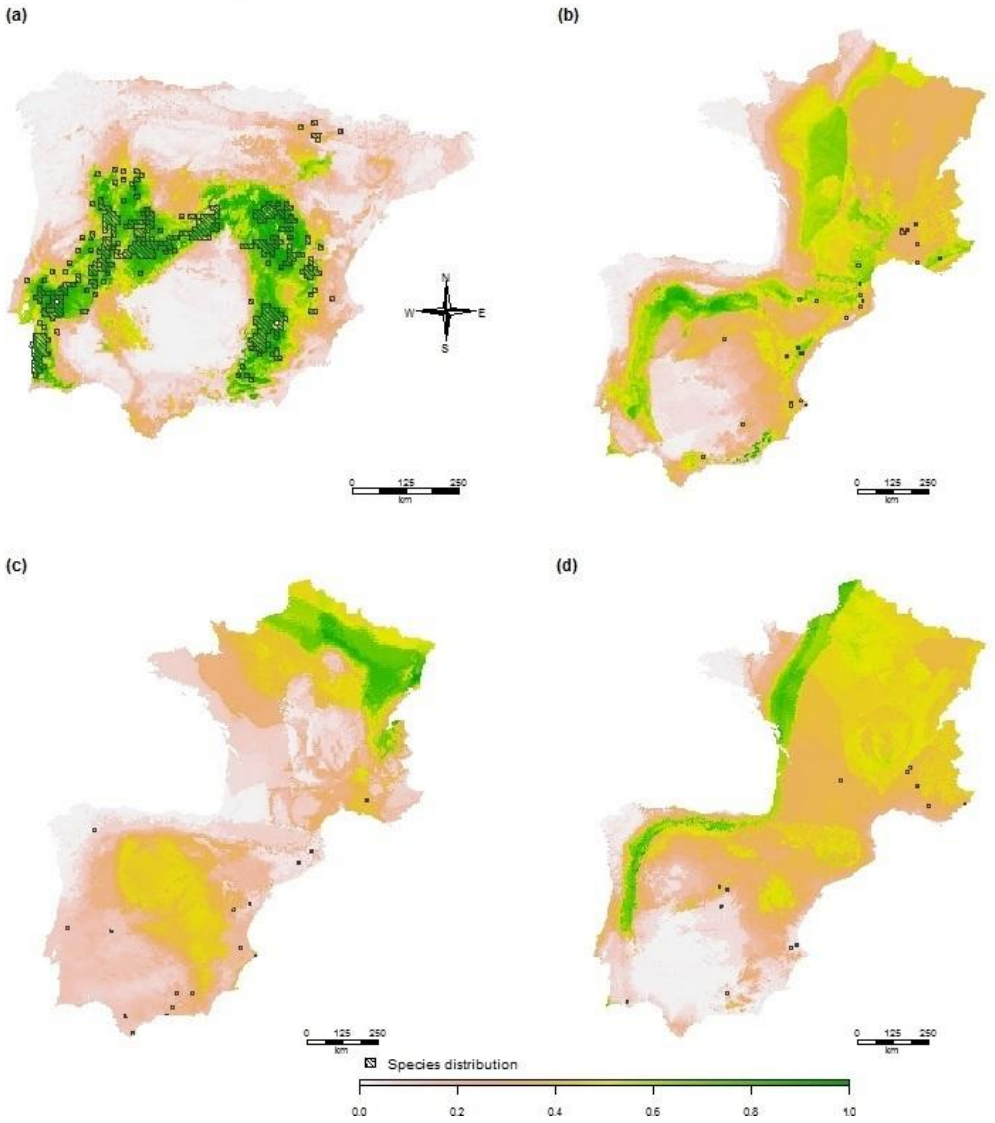

Figure 1. Random forest model projection to (a) current, (b) Mid-Holocene, (c) Last Glacial Maximum and (d) Last Interglacial Period climatic scenario. The shaded areas represent current occurrences (a) or fossil record of *M. cabrerae* for each epoch (b, c, d). Model is calibrated with the current distribution, thus it is restricted to the Iberian Peninsula, however the paleo-distribution included also France territories.

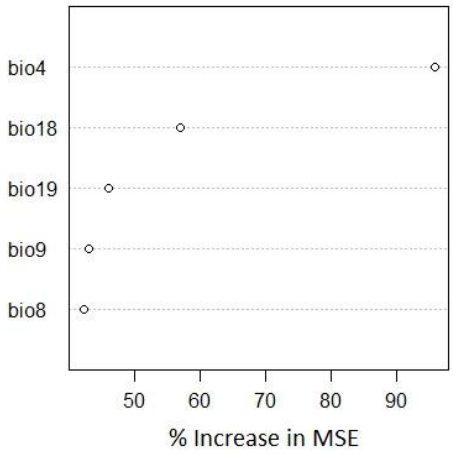

Figure 2. Importance of bioclimatic variables used in the random forest SDM from the geographical approach (R function *varImpPlot*, library randomForest; Liaw and Wiener, 2002). The percentage of increase in mean square errors (MSE) of model predictions is collected for each variable when that variable is randomly permuted, hence its importance in the accuracy of the model. Bioclimatic variables: temperature seasonality (bio 4), mean temperature of the wettest quarter (bio 8), mean temperature of the driest quarter (bio 9), precipitation in the warmest quarter (bio 18) and precipitation in the coldest quarter (bio 19).

In contrast, the transferability of the models to past conditions revealed that the most appropriate areas for the Cabrera voles were the north-eastern strip under Atlantic influence, Northern France and some territories under the effect of the Mediterranean Sea, depending on the epoch (Fig. 1b-d). These areas of high climatic suitability had the same range of temperature seasonality as that preferred by the species in its current niche. However, when temperature seasonality exceeded the calibration thresholds of the current model (Supplementary Fig. S1), species suitability in the paleo-scenarios was extrapolated to minimum values (according to the current biological response of the species). The most MoD showed the areas with non-analogous conditions in the Mid-Holocene, and especially in LIP (Supplementary Fig. S3). Therefore predictions in these areas would be speculative. Conversely, MoD revealed analogous environmental conditions between LGM and current climate scenarios. Beyond these non-analogous areas, hindcastings made some inaccurate predictions, such as the identification of northern France as the niche of this Mediterranean species during the coldest period (LGM).

The cross-projections of algorithms (Table 2) followed a similar trend to the models calibrated under current conditions and projected to the past. In general, the models calibrated with the

pseudoabsence dataset had higher AUC values in the internal evaluation (range: 0.813-0.958 versus 0.749-0.860 of the background dataset). However, independent validations with current occurrences reflected poor transferability in projections from the past to the present, as they were no different from random projections.

Table 2. Cross-projections. Modelling algorithms were calibrated in each paleoclimatic scenario and evaluated with AUC from the bootstrapping process (AUC of calibrated models). The evaluation of transferred models from paleo-scenarios to current climate was measured with AUC values using current occurrences as independent points (AUC of projection to current climate).

Model	Dataset	AUC (Calibrated Models)			AUC (Projection to Current Climate)		
		Mid-Holocene	LGM	LIP	Mid-Holocene	LGM	LIP
GLM	pseudo	0.925	0.813	0.816	0.599	0.553	0.589
RF		0.955	0.899	0.958	0.560	0.561	0.593
Maxent	bg	0.801	0.799	0.749	0.491	0.601	0.597
GLM		0.860	0.799	0.766	0.578	0.566	0.587

pseudo: pseudoabsences; bg: background

Climatic niche using an environmental approach

The global climatic space of the four scenarios (current, Mid-Holocene, LGM and LIP) was compiled through two PCA axes (Fig. 3). The variables of precipitation and temperature of warmest and driest quarter explained 33.9% of the variability, while the precipitation of coldest quarter, the temperature seasonality and the mean temperature of wettest quarter summarized 33.2% of the variability. The climatic space of Mid-Holocene and the current scenario was collected in Fig. 3a. The niche of the species under the Mid-Holocene climate occupied areas with higher precipitation in the warmest quarter and lower precipitation in the coldest quarter. Similar results were obtained in LIP (Fig. 3c). Niche stability was especially high in LIP with a value of 98% (Table 3); while the expansion ability of Cabrera voles under the warmer scenario was reduced to minimum values (2%, Table 3). On the other hand, LGM showed low stability in the environmental niche (55%, Table 3) and a niche expansion (45%, Table 3), indicating that *M. cabrerae* could have expanded its niche to a novel climatic framework. In LGM (Fig. 3b), the

realized niche had higher mean temperature and precipitation during the driest and the coldest quarter, respectively, lower values of temperature seasonality and lower precipitation in the warmest quarter.

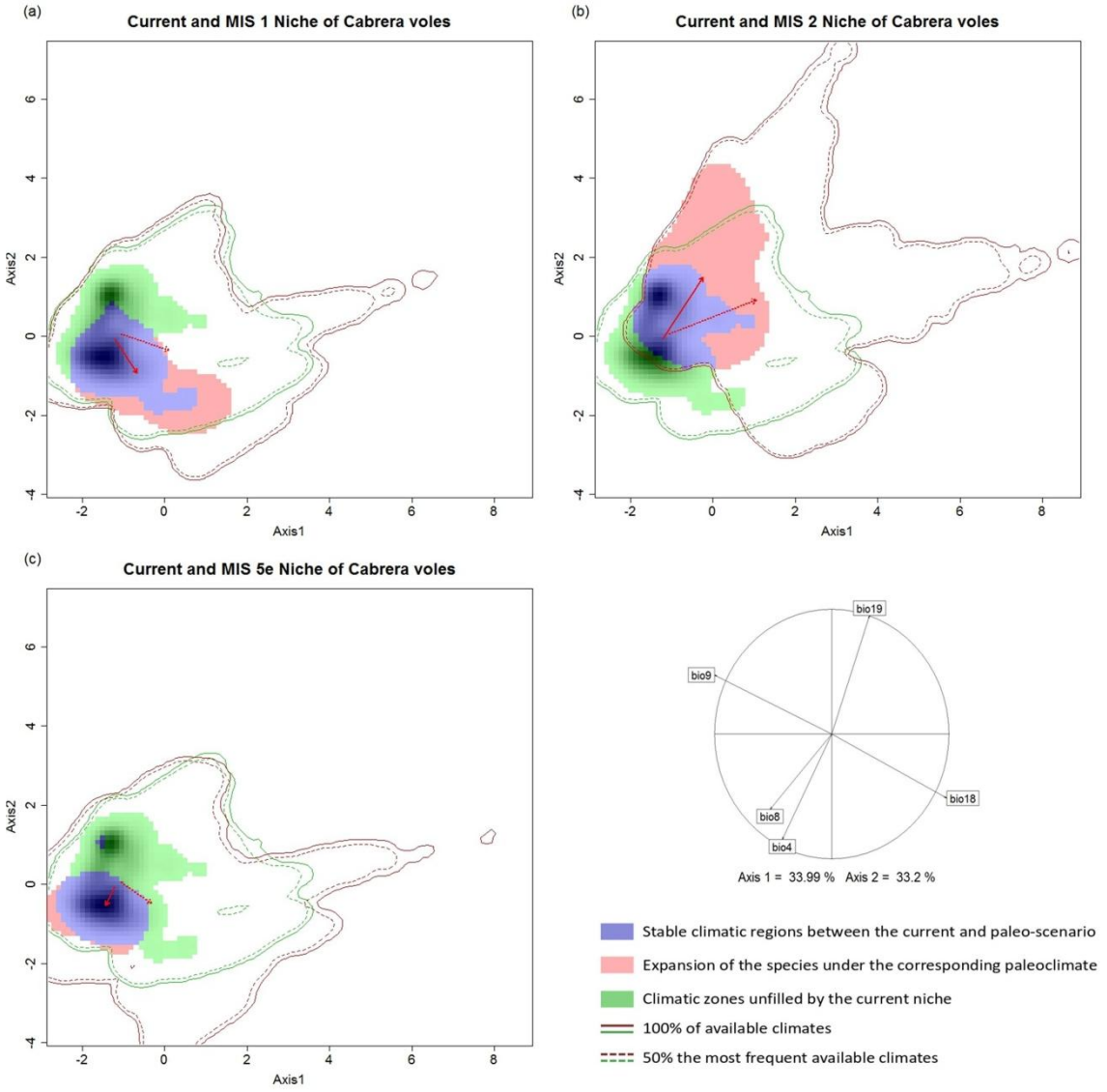

Figure 3. Climatic occupancy of *M. cabrerae* under Current and Paleo-climate: (a) MIS1 (Mid-Holocene); (b) MIS2 (LGM) and (c) MIS5e (LIP) periods. The arrows visualize the shift of the niche centroids between the current and paleo-scenario: continuous lines represent the shift in the centroid of current and paleo-distribution, while the dashed line is related to current and paleo extent. The correlation circle collects the contribution of bioclimatic variables to PCA axes.

Table 3. Niche overlap comparisons between current and paleo- scenarios of *M. cabrerae*. The statistical significance of niche overlap to evaluate the hypothesis of niche equivalency and the hypothesis of niche similarity were conducted with randomization tests. For niche equivalency, a significant result implies that the overlap between the current and the paleo-scenario is less than would be expected if niches were identical. The significance for similarity tests means that current and paleo-niches are more similar than would be expected from a random distribution of the species created inside the paleoclimate-scenario (CC-Paleo) or created inside the current climate (Paleo-CC).

Scenario	**Niche Overlap**	**Overlap index**			**Equivalency test**	**Similarity test**	
	D	Expansion	Stability	Unfilling		CC to Paleo-	Paleo- to CC
CC - Mid-Holocene	0.166	0.257	0.743	0.316	**	*ns*	*ns*
CC - LGM	0.151	0.451	0.549	0.169	**	*ns*	**
CC - LIP	0.480	0.019	0.981	0.312	**	**	**

CC: Current Climate

ns (non-significant): $p > 0.05$; *: $p \leq 0.05$; **: $p \leq 0.01$

Concerning the overlap of the environmental niche of paleo-scenarios and the current niche, none of the assessed paleo-niches were climatically equivalent to the current realized niche, as it is shown by the low Schoener's D values obtained ($p < 0.05$; Table 3). The results of the similarity test were not homogeneous throughout the three paleo-scenarios (Table 3). Since the similarity hypothesis could not be rejected under the Mid-Holocene scenario, niche overlap between the two temporal settings could be explained by regional similarities. Under the Current Climate-LGM scenarios, this hypothesis could only be partially rejected, as the paleo-niche of the species under cold LGM was not as similar to the current one as expected based on the environmental similarity between the two periods. However, this pattern was not observed from current climate to LGM scenario. Despite the non-equivalency between current and LIP niches, both were more similar to each other than would be expected by chance.

Discussion

The geographical and environmental assessments of the niche of the Cabrera vole illustrated a shift in its realized niche throughout its history. From an environmental perspective, the analysis revealed a lack of equivalency between current and paleo- niches, and therefore, reciprocal niche predictions from either range were unsuccessful. On the other hand, the similarity analyses clarified the response of *M. cabrerae* to the succession of climate changes from the Late Pleistocene until the present, indicating the expansion of the species' niche to novel climatic conditions. Thus, subtle variations in the Mid-Holocene climate were enough to drive changes in both the niche centroid and the niche envelope. Consequently, the LGM led to a major niche shift and the species was pushed towards areas of marked Mediterranean conditions. Laplana and Sevilla (2013) identified a contraction in the geographical distribution of the species with a movement towards lower latitudes, which supports our results of a shift to wetter and warmer territories. Conversely, even though the niche during LIP was not identical, the hot climatic conditions make the realized niche in that period more similar to the current niche than the surrounding locations. LIP was characterised by an average increase in temperature of 3.8º C and an average decrease in precipitation of 16% with current climate during the driest and warmest quarters. These arid conditions promoted a restricted realized niche compared to the current one. Assessments of the current status and threats to Cabrera vole populations have found that Mediterranean summers, with their high temperatures and low or no rainfalls, impose high restrictive conditions which act as a bottleneck, reducing population densities of this small mammal (Castellanos-Frías *et al*., 2015; Fernández-Salvador, 1998; Fernández-Salvador *et al*., 2005; Pita *et al*., 2006). Such climatic conditions of the current and Last Interglacial Period could be close to the species' physiological limits (Castellanos-Frías *et al*., 2015; Mathias *et al*., 2003). These conditions could have relegated the species' niche to specific habitats which could ensure the necessary environmental requirements that could not be satisfied by climate (*e.g.* areas of high phreatic level or areas in mountain foothills) (Garrido-García and Soriguer-Escofet, 2012; Laplana and Sevilla, 2013).

From the geographical approach, SDM transferabilities were not able to locate the realized niche of *M. cabrerae* for any of the scenarios based on the fossil record. The main drawback in these temporal transferabilities is the non-analogue conditions between the current and paleoclimatic scenarios for some variables like temperature seasonality. Even though temperature seasonality values fall outside the calibration ranges, it is a very important variable as shown by both by the SDMs and the biology of the species (Fernández-Salvador *et al*., 2005), and, therefore, its use is

justified. Excluding these speculative predictions, the suitable areas predicted by hindcasting are very distant from the fossil record. Furthermore, the inaccurate current predictions from the SDMs cross-projections support that the relationship of the species with the environmental variables could have changed. This shift could have affected the set of bioclimatic variables defining the realized niche of Cabrera voles, as detected in some biological invasions of plants (Broennimann *et al*., 2007) and temporal transferabilities of mammals (Rubidge *et al*., 2011). The use of additional biotic variables such as habitat type, degree of fragmentation of the territory or distribution of other species could have improved SDM transferability (Wogan, 2016). However, these variables would have to be estimated from extrapolated data for the paleo-scenario, which would make the transferability of SDM projections less accurate. Despite using a well-calibrated and complete fossil record database, the use of fossil records can result in potential biases, as the records may not be representative of the species' distribution. For instance, most Cabrera vole fossils in karsts of calcareous formations are from mountains of alpine origin, and there has traditionally been less interest in the study of small mammals (Laplana and Sevilla, 2013). In addition, the number of records from the Last Pleistocene sites is quantitatively lower than in those with recent chronology (Laplana and Sevilla, 2013). This could lead to inaccurate predictions of the species paleo-niches, and these limitations could be skewing our results.

Fløjgaard *et al*. (2009) and Vega *et al*. (2010) used SDM transferability to properly identify the niches of a set of North-Central European small mammals during the LGM, assuming niche conservatism. Conversely, McGuire and Davis (2013) inferred a niche shift in three out of five evaluated *Microtus* species since the last notorious climate change, the LGM in North America. Likewise, Guralnick and Pearman (2009) identified a change in the niche of two small mammals species from flatland territories, while a set of mountainous species did not show a niche shift. However, the lack of transferability of the SDMs of five mammal species to the North American LGM led Davis *et al*. (2014) to reject the flatland-mountain hypothesis. Accordingly, studies on microtines have revealed varied responses and grades of niche conservatism dependent on taxa, environmental context or the temporal-frame (Peterson, 2011).

The poor performance of SDM transferability has generally been interpreted to support niche shift (Broennimann *et al*., 2007; Davis *et al*., 2014; Guralnick and Pearman, 2009; McGuire and Davis, 2013; Worth *et al*., 2014). The grounds for these niche shifts may be mainly attributed to some adaptation ability, a truncation of species' fundamental niche or the absence of equilibrium between the species' distribution and climate (Guisan *et al*., 2012; Maiorano *et al*., 2013; Pearman *et al*., 2008; Veloz *et al*., 2012). Microtines are a highly diverse group of

mammals with high karyotypic variability and a rapid evolution rate, contrary to what could be expected due to their phenotypic homogeneity (Martin and Palumbi, 1993; Megías-Nogales *et al*., 2003; Triant and DeWoody, 2006). According to Triant and DeWoody (2006), new *Microtus* species may evolve every 30,000 years; consequently, changes in their fundamental niches could be expected. However, the identification of such adaptive processes requires mechanistic approaches with experimental measurements (Guisan *et al*., 2012). Our methodological framework is unsuitable to rule out phenotypic plasticity, as a consequence of the truncation of the fundamental niche of the species. The niche truncation involves a hiding of the species' range to climatic tolerance (Feeley and Silman, 2010); thus, it may explain the expansion to novel climatic conditions of the current and paleo- realized niches. Despite considering several factors, Veloz *et al*. (2012) pointed out truncation as the most probable cause to explain the failure in current niches predictions of fossil-pollen data from 21 to 15 ka BP. In fact, these authors conclude that the realized niche only covers a temporal subset of the suitable environmental conditions of the taxon. Likewise, shifts in niches may be caused by changes in positive or negative biotic interactions or in dispersal limitations (*i.e.* lack of species-climate equilibrium (Nogues-Bravo, 2009)) of *M. cabrerae* since the Late Pleistocene. Pearman *et al*. (2008) revealed the importance of biotic interactions as modeller of the realized paleo-niche of some plant species (larger influence than climate). Some authors consider that this lack of success could also be attributed to other factors such as excessive model complexity or overfitted models (Maguire *et al*., 2016; Peterson, 2011), inaccuracies in paleoclimate reconstructions (McGuire and Davis, 2013), vagueness in fossil dating (Davis *et al*., 2014) or not considering other factors than temperature and precipitation (Davis *et al*., 2014; Wogan, 2016). Part of the SDM's limitations could be overcome using a wider approach based on CLMs (Maguire *et al*., 2016) or multi-temporal approaches to estimate fundamental niches (Maiorano *et al*., 2013). CLMs solve the model overfitting because they include multi-taxa data, which provides a better prediction in non-analogue climates or communities (Maguire *et al*., 2016). On the other hand, the vagueness in fossil dating requires updating in the SDM when its chronology is clarified.

Under the current timeframe, all SDMs had good performance and properly located the occurrences of the species. According to Garrido-García *et al*. (2013), the distribution of *M. cabrerae* is arranged in four unconnected population cores. However, our models found high climatic connectivity between occurrences, which may be due to some biotic interaction or anthropogenic disturbance. Spatial transferability beyond current distribution limits reveals areas of climatic suitability in south-eastern France, even though the species is not currently

distributed there. The fossil record shows that Cabrera voles inhabited this territory from the Late Pleistocene until quite recent times, as the last records in France dated from *ca.* 1900-2000 years BP (Poitevin *et al*., 2005). Previous studies have reported a historic contraction in the distribution of the species and its disappearance from France as a result of climate, interspecific interactions and/or an intensification of anthropogenic impacts (Garrido-García and Soriguer-Escofet, 2012; Laplana and Sevilla, 2013; López-García and Cuenca-Bescós, 2012). Nevertheless, the results suggest that climate is not the main cause of extinction in the French territory. Furthermore, the Pyrenees mountain chain was not an impassable barrier for the species as it was not for similar microtines of northern latitudes like *Microtus oeconomus*. The fossil record shows that this species crossed the Pyrenees during cold LGM from northeastern Europe and reached the Central mountain range of the Iberian Peninsula (Laplana and Sevilla, 2013), searching for a suitable climate. So, even though the less favourable climate could explain the contraction in Cabrera vole distribution, we consider the species might be able to recover a territory with current climatic suitability, as it did during the events of past climate change. On the other hand, southeastern France is currently inhabited by *Microtus agrestis*, *Microtus arvalis* and *Arvicola sapidus*, all living in moist habitats with rich grass cover, although *M. arvalis* is mainly associated to agricultural lands (Mitchell-Jones *et al*., 1999). Specifically, *A. sapidus* is deemed a competitive species which can displace the Cabrera vole because of its larger size (Pita *et al*., 2006). However, based on its current distribution and the fossil record, the two species seem to have segregated the spatial and temporal use of habitats (Fernández-Salvador, 1998; López-García and Cuenca-Bescós, 2012; Pita *et al*., 2011). Therefore, *A. sapidus* is not considered to be responsible for competitive displacement in France. Conversely, interspecific competition with the other microtines for a habitat specialist species like *M. cabrerae* could explain why Cabrera voles are not in the French territory. Consequently, biotic interactions may be having a large influence on the species' current realized niche and thus, it could also be the case for the paleo-niches.

In conclusion, a combined environmental and geographical framework has enabled us to identify the lack of niche conservatism in the Cabrera vole. Therefore, forecastings of climate change should contemplate the non-niche conservatism approach and integrate other mechanisms of species, such as adaptation or phenotypic plasticity, rather than only global geographical migration towards higher latitudes. Based on our geographical approach, the species' distribution should be limited to northern territories. However, the fossil record does not support these predictions. Our historical assessment clarifies the capabilities and limitations of this Iberian endemic vole from its own trajectory. Thus, *M. cabrerae* may have revealed some

degree of adaptation to deal with climate changes throughout the wide time periods. Nevertheless, the adaptive ability of the species could be highly limited under arid conditions like LIP where the species reduced its environmental space notoriously. Therefore, a future climate change scenario developed in less than a century with warm temperatures like in LIP but drier might exceed the adaptive ability of Cabrera voles and range contraction could be the general rule. Notwithstanding, the realized niche shift could be also attributed to the non-equilibrium of species' distribution with climate and the truncation of its fundamental niche. These alternatives should be analyzed in future studies using other approaches.

Supplementary Information

Supplementary Fig. S1. Response curves of *M. cabrerae* to bioclimatic variables in its current distribution.

Supplementary Fig. S2. Suitability map of the spatial transferability of current SDM (Random forest model) for *M. cabrerae* beyond its distribution limits.

Supplementary Fig. S3. Most dissimilar variable analysis (MoD) of Maxent software across the three paleoclimatic scenarios.

Supplementary Fig. S4. Average values of the bioclimatic variables.

Supplementary Table S1. Fossil record of *Microtus cabrerae* derived from Laplana and Sevilla (2013) and completed with Cuenca-Bescos *et al*. (2014).

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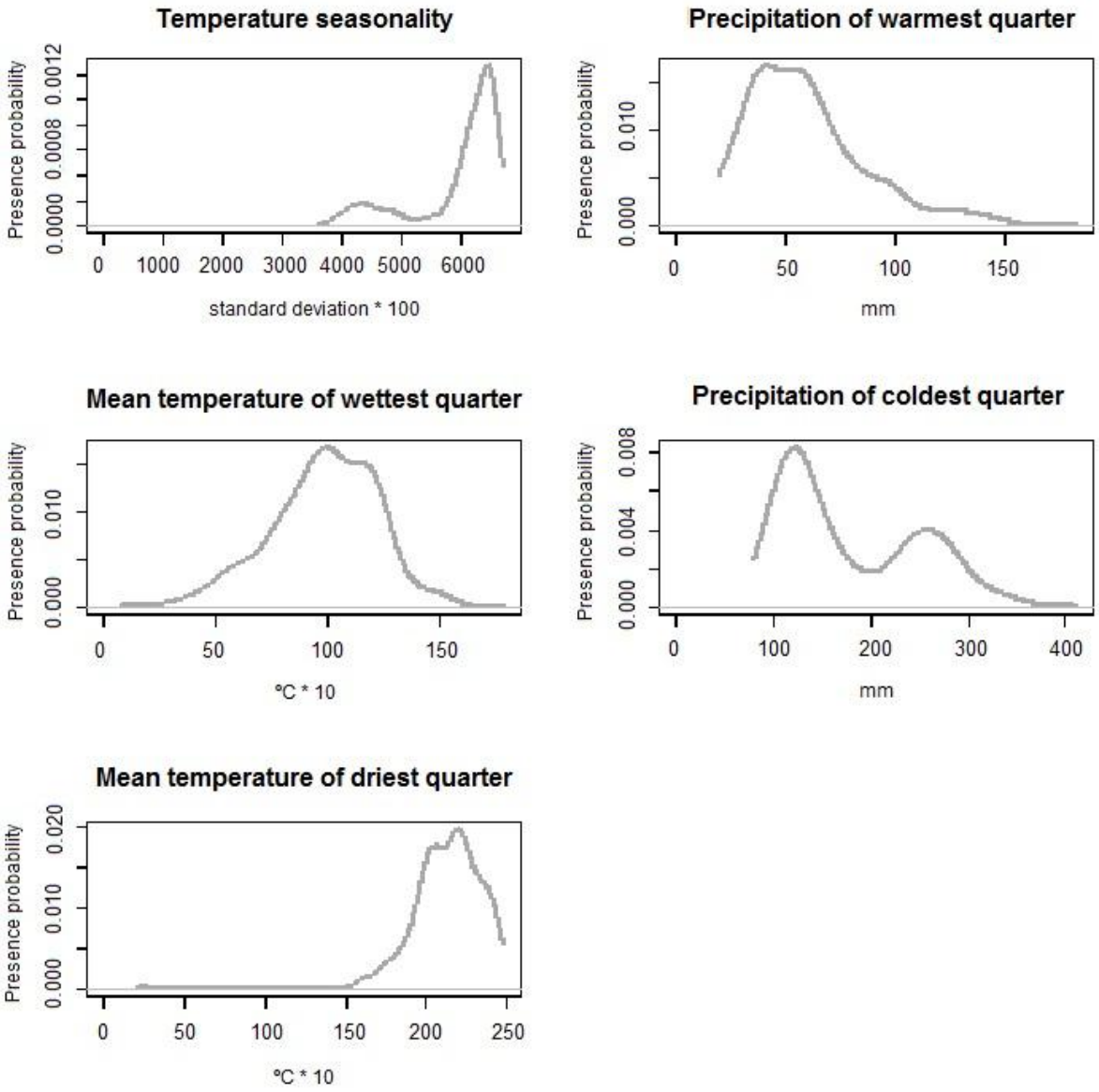
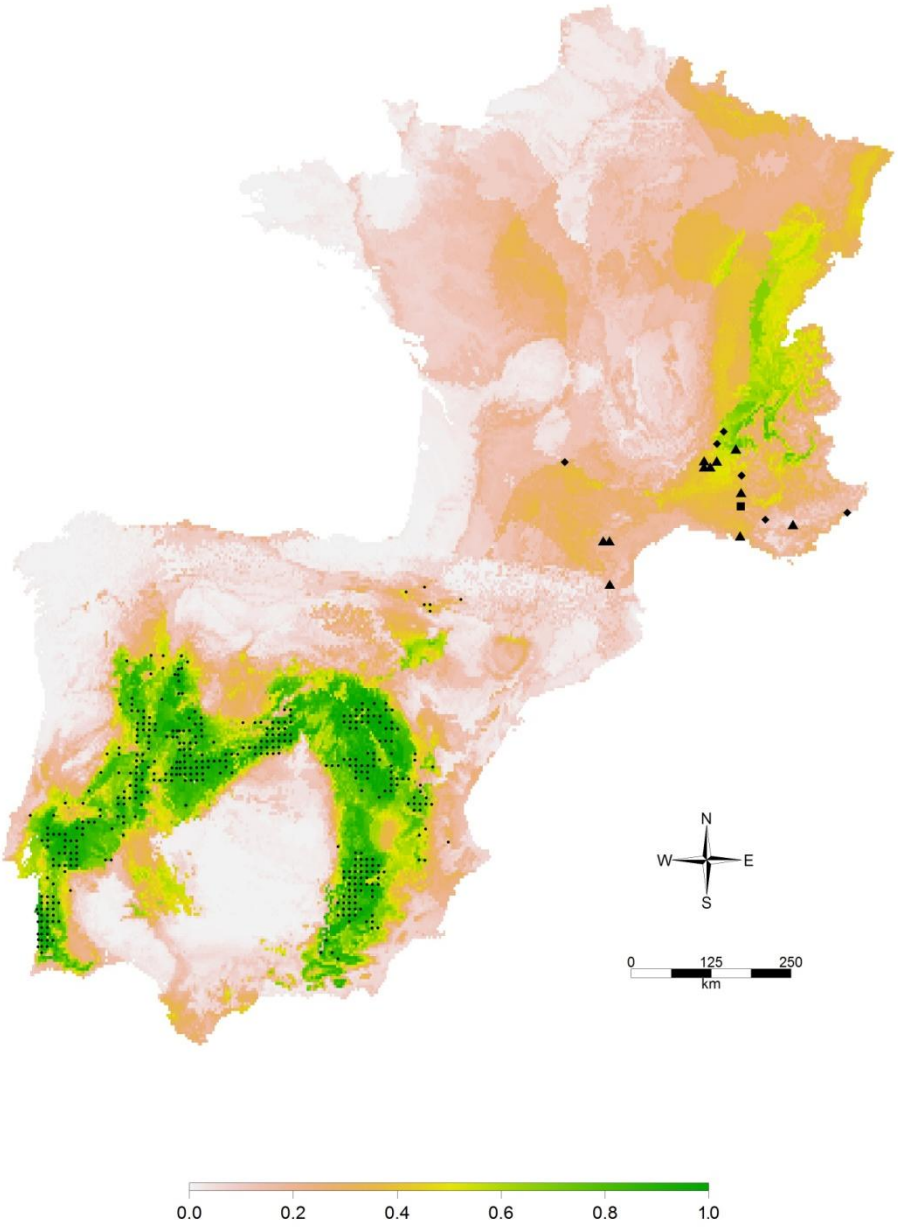
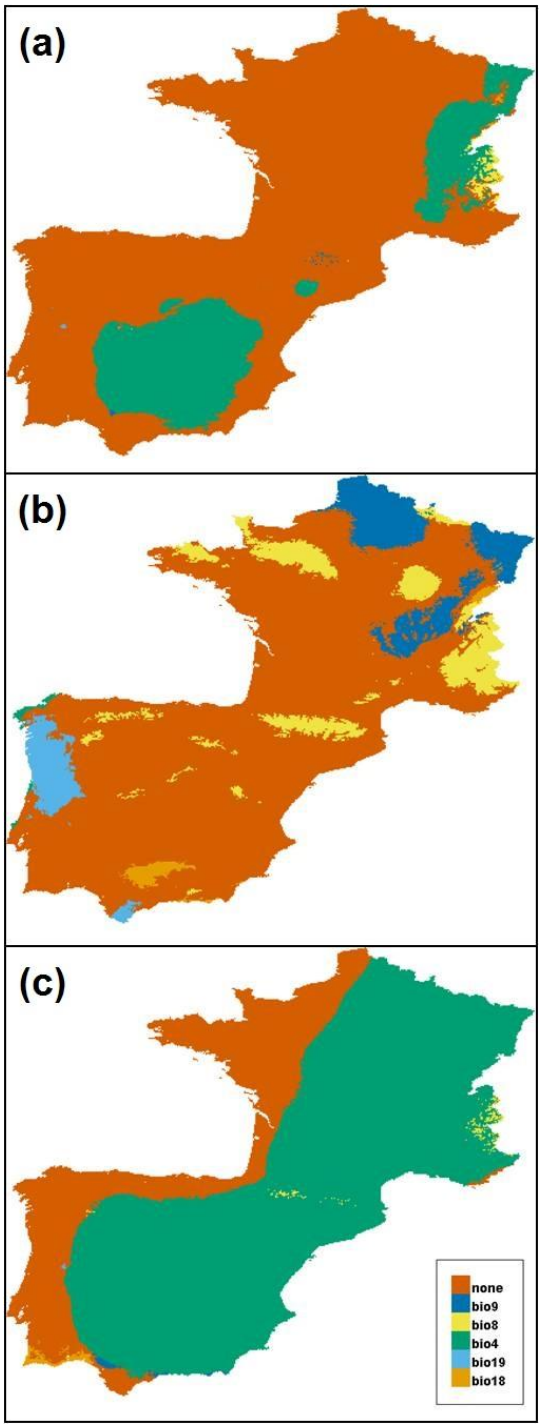
Supplementary information



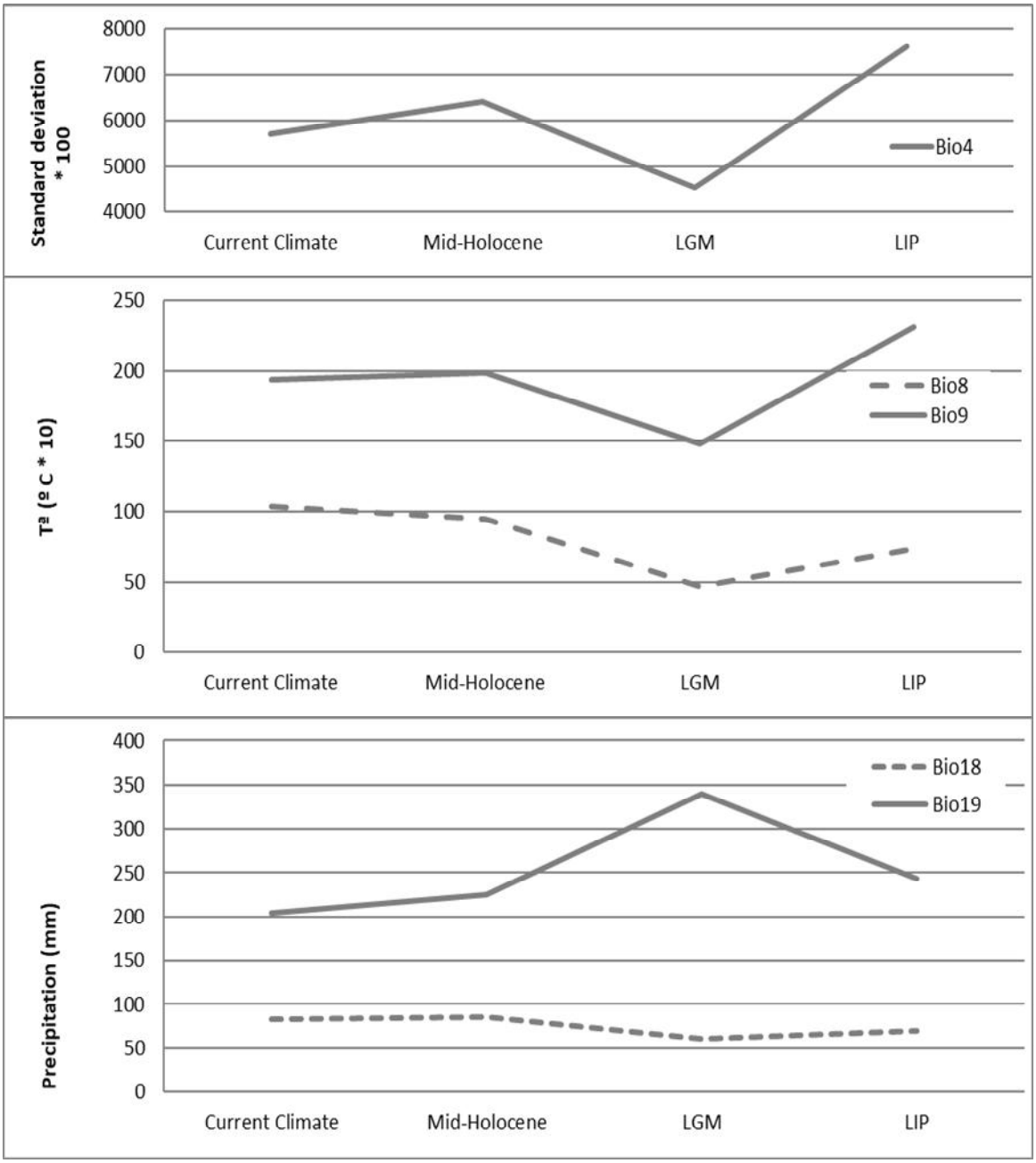
Supplementary Fig. S1. Response curves of *M. cabrerae* to bioclimatic variables in its current distribution.



Supplementary Fig. S2. Suitability map of the spatial transferability of current SDM (Random forest model) for *M. cabrerae* beyond its distribution limits. Dots identify the (●) current occurrences, the fossil record of (▲) Mid-Holocene, (■) Last Glacial Maximum and (◆) Last Interglacial of the species.



Supplementary Fig. S3. Most dissimilar variable analysis (MoD) of Maxent software across the three paleoclimatic scenarios: (a) Mid-Holocene, (b) LGM and (b) LIP. The bioclimatic variables indicated in the maps represent areas where the values of the variable are outside the range used during model calibration. Model projection in these areas is executed keeping the values of climatic suitability as threshold values in the calibration.



Supplementary Fig. S4. Average values of the bioclimatic variables: temperature seasonality (Bio 4), mean temperature of the wettest quarter (Bio 8), mean temperature of the driest quarter (Bio 9), precipitation in the warmest quarter (Bio 18) and precipitation in the coldest quarter (Bio 19) for the four climatic scenarios (Current climate, Mid-Holocene, LGM and LIP) in the common range of the Iberian Peninsula.

Supplementary Table S1. Fossil record of *Microtus cabrerae* derived from Laplana and Sevilla (2013) and completed with Cuenca-Bescos *et al*. (2014). Chronological filtering was performed on the original fossil record set to select the records within the considered climatic chronology.

Period	Country	Site	Chronology (ka BP)	Reference
Mid-Holocene	France	Combe Obscure	*ca*. 4.5 - 6.5	Brandy (1977); Helmer *et al*. (2005)
		Grotte de la Chauve-Souris	*ca*. 3.845 - 9.94	Jeannet and Vital (2009)
		Baume d'Oullins	*ca*. 4.5 - 6.5	Helmer *et al*. (2005); Helmer and Vigne (2007)
		Grotte de Fontcombe		Jeannet (1988)
		Baume Claire and Baume Sourde	*ca*. 3.95 - 5.3	Jeannet (2000); Furestier (2005); Ducos (1958); Jeannet and Vital (2009)
		Abri de la Font-des-Pigeons		Ducos (1958);Poitevin *et al*. (1990)
		Baume de Font-Brégoua		Brandy (1977); Brandy (1976); Poitevin *et al*. (1990); Paunesco and Abbassi (1999); Paunesco and Brunet-Lecomte (2005)
		Grotte d'Unang	*ca*. 4 - 6	Brandy (1976); Michaux (1993); Hervet (2000)
		Grotte de l'Abeurador	*ca.* 6.26 ± 0.09	Marquet (1987); Marquet (1993); Vaquer and Ruas (2009)
		Abri de Font-Juvénal	*ca*. 4.2 - 6.41 ± 0.1	Marquet (1987); Thiebault and Vernet (1992); Marquet (1993)
		Caune Ouest	*ca*. 5.165 ± 0.125	Brochier *et al*. (1998); MuséumNationald'HistoireNaturelle (2003-2012)
	Spain	Cova del Frare	*ca*. 4.45 - 6.38	Alcalde Gurt (1986); Albert *et al*. (1997)
		Cova del Pasteral	*ca*. 5.27 ± 0.07	Alcalde Gurt (1986); Gibaja *et al*. (2010)
		Cova 120	*ca*. 3 - 6	Alcalde Gurt (1986); Agustí *et al*. (1987); Cucchi *et al*. (2005); Cuenca-Bescos *et al*. (2014)
		Cova de l'Arbreda		Alcalde Gurt (1986); Alcalde Gurt (1987)
		Cova Colomera	*ca*.6.87 - 7.06 ± 0.07	Bañuls-Cardona and López-García (2009); López-García and Cuenca-Bescós (2010); López-García *et al*. (2010a)
		Cueva del Coscojar		Gil Bazán (1985); Fernández-Posse *et al*. (1996)
		Cueva de Chaves	*ca*. 6.12 - 6.77 ± 0.07	Utrilla *et al*. (2008)
		Cova de les Cendres	*ca*. 6 - 7.5	Guillem Calatayud (1995); Guillem Calatayud (1999, 2001); Guillem Calatayud (2009)

		Cova de Bolumini		Guillem Calatayud (1995); Guillem Calatayud (1999)
		Abric de la Falguera	*ca*. 6.51 ± 0.07	Guillem Calatayud (2006); Puchol and Tortosa (2006)
		Cova de la Sarsa-Sector II: Gatera	*ca*. 6.9	López-García and Cuenca-Bescós (2012); Sevilla (1988)
		Abric del Mas Martí	*ca*. 6.5	Fernández López de Pablo *et al*. (2005)
		Cingle del Mas Cremat	*ca*. 6.74 - 6.99	Guillem Calatayud (2010); Vicente Gabarda *et al*. (2009)
		Cova Fosca	*ca*. 6.2	Sesé (2011)
		Cueva de la Ventana	*ca*. 6.01 - 6.56 ± 0.04	Doce *et al*. (2012); Sánchez *et al*. (2005)
		Cueva de El Toro	*ca*. 4.22 - 4.33	Mederos Martín (1996); Watson *et al*. (2004)
		Cueva del Nacimiento	*ca*. 4 - 6	López Martínez and Sanchiz (1981)
LGM	France	Abri de Soubeyras	*ca*. 15.075 - 18.27	Brochier (1977); Crégut-Bonnoure *et al*. (2014)
	Spain	Cova de l'Arbreda	*ca*. 19.48	Alcalde Gurt (1986, 1987); Alcalde Gurt *et al*. (1981)
		Cueva de Valdavara I	*ca*. 17.89	Bañuls-Cardona *et al*. (2014); López-García *et al*. (2011a); Vaquero Rodríguez *et al*. (2009)
		El Portalón	*ca*. 16.89 - 30.3	Bañuls-Cardona *et al*. (2014); López-García (2011); López-García *et al*. (2010b)
		Cova de les Cendres	*ca*. 21.23	Guillem Calatayud (1995); Guillem Calatayud (2001); Villaverde *et al*. (1997); Villaverde and Roman (2004); Villaverde *et al*. (2010)
		Cova Negra	*ca*. 21	Guillem Calatayud (1995); Guillem Calatayud (2001)
		Sala de las Chimeneas	*ca*. 21.63 - 21.27	Bañuls-Cardona *et al*. (2014); Bañuls and López-García (2010); Bañuls *et al*. (2012)
		Cueva de la Carihuela	*ca*. 18.8 - 22.4	Fernández *et al*. (2007); Ruiz Bustos (2000)
		Higueral de Valleja Cave	*ca*. 20.78	Jennings *et al*. (2009); Turner *et al*. (2008)
		Cueva de la Pastora I		Ruiz Bustos (1996, 2007)
		Cueva de los Ojos	*ca*. 18 - 22	Fuentes Jiménez (1989)
		Cueva de Nerja	*ca*. 17.5 - 24	Cortés-Sánchez *et al*. (2008)
		Cova del Toll	*ca*. 13 - 35	Fernández-García (2014); Fernández-García and López-García (2012)
		Cova Fosca	*ca*. 19	Alcalde Gurt (1986); Cuenca-Bescos *et al*. (2014)
	Portugal	Grotte de Caldeirao	*ca*. 18 - 30	Póvoas (1991); Povoas *et al*. (1992)

	United Kingdom	Gorham's Cave	*ca*. 18.4	Cuenca-Bescós *et al*. (2007); Denys (2000); López-García *et al*. (2011b); Price (2012)
LIP	France	Grotte de Payre	*ca*. 105 - 135	Desclaux *et al*. (2008); Rivals *et al*. (2009)
		Baume Moula Guercy	*ca*. 100 - 120	Defleur *et al*. (2001); Defleur *et al*. (1999); Desclaux and Defleur (1997)
		Grand Abri aux Puces	*ca*. 117 - 127	Slimak *et al*. (2010)
		Grotte de l'Adaouste		Defleur *et al*. (1994)
		Grotte de Le Lazaret	*ca*. 130	Hanquet *et al*. (2010); Valensi *et al*. (2007)
		Coudoulous II	*ca*. 112 ± 35 - 140	Cochard (2004); Couchoud (2006)
	Spain	Cova de Bolomor	*ca*. 121 ± 18	Fernández Peris *et al*. (2008); Guillem Calatayud (1995); Guillem Calatayud (2001)
		Cova Negra	*ca.* 117 ± 17	Arsuaga *et al*. (2007); Fernández Peris *et al*. (2008); Guillem Calatayud (1995); Guillem Calatayud (2001); Perez Ripoll (1977)
		Cueva de las Pinturas		Sesé and Ruiz Bustos (1992)
		Cueva del Camino	*ca*. 74 - 140	Arsuaga *et al*. (2010); Arsuaga *et al*. (2011); Arsuaga *et al*. (2012); Blain *et al*. (2014); Laplana and Sevilla (2006); Toni and Molero (1990)
		Preresa	*ca*. 157 ± 46	Moreno *et al.*, 2018
		Cueva de la Carihuela		Ruiz Bustos (2000); Vega Toscano (1999)
	Portugal	Goldra	*ca*. 125	Antunes *et al*. (1986)

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Capítulo 4

Is the niche preserved in the Arvicoline species from the Holarctic?

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Manuscrito en preparación.

Abstract

The effort to clarify the biological and physical motivations underlying the species' distribution patterns has led to study the niches and its dynamics. The investigation into niches is usually dominated by the niche conservatism assumption (*e.g.* through of Species Distribution Models), in which it is considered that species retain their ancestral ecological characteristics in time and space without including adaptive processes. However, the literature has identified in many taxa niche shift patterns, so it is necessary to enquire into the taxa's niche before considering general assumptions.

Phylogeny on niche studies provides an alternative approach to reveal similarity between related species (phylogenetic niche conservatism, PNC) or differences (phylogenetic niche divergence, PND). Therefore, using a methodological approach based on the niche overlap measurement and niche dynamics tests we evaluated the conservatism of niches throughout the Arvicoline phylogeny. Complementarily, the correlation between phylogenetic and environmental distances was assessed through the Mantel test. All the pairs of species had a niche overlap lower than the 50% and in only four pairs this overlap was significant. Hence, both approximations revealed a general rule of non-niche conservatism throughout the phylogeny. In sum, most of the species of the subfamily Arvicolinae may have suffered niche shifts, moving away from the conditions of the ancestral niche. Nevertheless, this trend cannot be assumed for the whole group.

Key words. Arvicoline; Rodents; Phylogenetic niche conservatism; Phylogenetic niche divergence; Niche overlap; Equivalency test; Similarity test; Mantel test; Niche dynamics.

Introduction

How species are distributed throughout the globe has been an age-old challenge issue which has encouraged many ecologists to enquire into the biological and physical motivations underlying the distribution patterns (Krebs, 1972; Wiens & Graham, 2005). The study of the processes behind such patterns has been analysed through different alternatives, but the use of the niche concept has been among the preferred (Wiens & Graham, 2005). In the early of 20th century, Grinnell (1917) coined the niche concept in terms of the abiotic factors of the environmental space, which was refocused towards the widely used Hutchinson's (1957) niche. The niche is conceived as a n-dimensional hypervolume where the species has a positive demographic growth rate at population level. In this vein, the space purely physic or abiotic concerns the fundamental niche, which only can be approximated with physiological experiments carried in field or laboratory (Guisan & Thuiller, 2005; Soberón & Arroyo-Peña, 2017). Biotic interactions and dispersion processes constrain niches in the realized niche (Pearman *et al*., 2008), which can be estimated with correlative methods from the species' occurrences (Guisan & Thuiller, 2005; Soberón, 2007).

Niches are commonly addressed from the assumption that the species retain their ancestral ecological characteristics (Wiens & Graham, 2005) in time and space (Petitpierre *et al*., 2012), preserving the fundamental and/or realized niches, that is, niche conservatism (Pearman *et al*., 2008). So that, the global change such as climate change, habitat destruction or biological invasions poses a high threat to biota with low adaptation ability (Wiens & Graham, 2005). On the contrary, some taxa could develop ability to adapt to changing conditions and, so show niche shifts, meaning, any change in the centroid or limits of the niche (Guisan *et al*., 2014). These niche shifts question the suitability of the niche-based models (such as Species Distribution Models, SDMs) to anticipate alterations in taxa's distributions, since the SDMs are based on the niche conservatism assumption (Pearman *et al*., 2008; Nogues-Bravo, 2009; Petitpierre *et al*., 2012). The change in any of the three BAM factors (biotic, abiotic or movement) would modify the species' niche and so would alter the SDM's predictions (Pearman *et al*., 2008). Therefore, the niche conservatism assumption may be altered by the adaptive ability of the taxa (Pearman *et al*., 2008; Stigall, 2014), but also the approach used in the study can drive to different conclusions, such as: the spatial-temporal scale (Nogues-Bravo, 2009; Peterson, 2011), the niche dimensions analysed (Peterson, 2011) or the level of biological organization (Stigall, 2014; Peixoto *et al*., 2017).

Phylogeny on niche studies provides an alternative approach to enquire into the degree of divergence of the niches from ancestral conditions (Pearman *et al*., 2008; Cooper *et al*., 2010; Wiens *et al*., 2010), in order to reveal patterns of similarity between related species (phylogenetic niche conservatism: PNC), or, on the contrary, marked differences in niches of closely related species (phylogenetic niche divergence: PND). The advantage of the use of the phylogeny lies in its independency of the niche conservatism assumption. Several methods allow approximate the controlling phylogenetic mechanism: the most often used are the Pagel's (1999) lambda metric and the *K*-statistic of Blomberg *et al*. (2003) to detect phylogenetic signals significantly greater or lower than the expected under a Brownian motion (Losos, 2008). However, a low degree of correspondence between PNC levels and the phylogenetic signal has been identified (Ackerly, 2009; Revell & Collar, 2009) using only these metrics (Pearman *et al*., 2014). Therefore, other tools such as the measurement of niche overlap of closely related species are also available to test the PNC and can provide new insights with which understand the patterns of niche conservatism (*e.g.* Warren *et al*., 2008; Aguirre-Gutiérrez *et al*., 2015). Through the comparison of the environmental space of the species of the phylogeny, it is expected that closely related species have more similar niches (closer to the ancestral niches) than other species of the phylogeny. Nevertheless, species living under similar environmental conditions may have similar niches, without having a direct phylogenetic relationship, hence the inclusion of species' spatial distribution on the study of PNC provides additional data to discern the niche similarities not attributable to the phylogeny (Cooper *et al*., 2011). So, the combined use of phylogeny and environmental data of the species can contribute to clarify the causes behind the niche shifts and the timescales in which they occur in order to explain the niche dynamics (Pearman *et al*., 2008).

In the framework of the study of PNC, the subfamily Arvicolinae (Mammalia, Cricetidae) poses an ideal study model because of its relative short time of divergence (*ca*. 2 to 3 million years ago) (Martínková & Moravec, 2012), which may be indicative of niche conservatism as a general rule in this young group. Despite this fact, the Arvicolinae is a subfamily of rodents which has experienced a relevant radiation throughout the Holarctic region (Robovský *et al*., 2008), actually it is one of the mammalian group with most species-rich (Martínková & Moravec, 2012). The geographic isolation of populations in refugia during the successive glacial periods has been pointed as a trigger for the quick diversification in Arvicoline (Bilton *et al*., 1998). Hence, species with reduced distribution ranges may often have led to differentiated groups (Martínková & Moravec, 2012). Therefore, the Arvicoline species are characterized by having a high genetic diversity with great karyotypic amplitude (Megías-Nogales *et al*., 2003; Jaarola *et al*., 2004)

though low phenotypic variability (Triant & DeWoody, 2006). Most of the species show preference for habitats such as forest, scrubland and grassland from the temperate zone, arable lands and pasturelands; however, other species do not discard habitats such as wetlands, tundra grasslands, boreal forests or subtropical forests (IUCN, 2018).

Considering the contrast of being a subfamily with a relative short time of divergence (hence the niche conservatism may be likely) and having a high diversification rate (which may be indicative of niche shifts), joined to the variety of habitats they can inhabit, we believe that the Arvicoline group is an interesting model to assess the PNC. As a consequence of the genetic and environmental peculiarities of this subfamily, we hypothesized that the high speciation rate might have led towards the non-niche conservatism in these rodents. Therefore, we aim to elucidate the grade of conservatism between the most closed species in order to identify the predominant pattern of niche conservatism or shift throughout the phylogeny. Specifically, the realized niches of the sister species were compared with an environmental approach to establish the niche overlaps, the equivalencies and the similarities between the Arvicolinae subfamily. Complementarily, the correlation between phylogenetic and environmental distances was evaluated using Mantel test.

Materials and methods

Arvicoline phylogeny

The evolutionary history of Arvicoline species from Holarctic region was addressed through the reconstruction of phylogenetic relationships proposed by Martínková and Moravec (2012). This phylogenetic hypothesis combines mitochondrial and nuclear DNA sequence data to establish the relation between taxa. Based on this phylogeny, some species had to be discarded because of the lack of distributional data: the analytical method did not run for species with lower than five occurrence points. Those species with insufficient occurrence points or without geographical information were suppressed from the analysis and substituted by the nearest sister species. Finally, a subset of 23 sister species pairs (detailed in Table 1) was used in the phylogenetic niche conservatism analyses.

Species records

Data occurrences of species were compiled from GBIF (GBIF.org, 2018) and IUCN (IUCN, 2018) databases through the function *gbif* (R library dismo; Hijmans *et al*., 2016) and ArcGIS 10.2.2 software, respectively. Information from GBIF was preferred over IUCN, because of the accuracy of geographical coordinates. A pre-processing of GBIF's occurrences was executed in order to remove duplicated points, data without geographical coordinates, fossil records or incomplete information. IUCN data were homogenized to GBIF format, so a point-grid of 0.61 decimal degrees (approx. 48 km) was created inside the species' distribution areas to locate their occurrence points.

Environmental variables

Abiotic predictors of the niches were integrated through bioclimatic variables from WorldClim database (Hijmans *et al*., 2005) with 5 min (approx. 9.3 km x 9.3 km) of spatial resolution. With the aim to minimize the multicolinearity, a cluster dendrogram with Pearson correlations was applied, so a single bioclimatic variable was selected from each branch (Pearson correlation: $r < \pm 0.7$) according to a biological criterion. By each species it was evaluated the most important variables in order to form a common set of variables for all species. Finally, the temperature seasonality variable (Bio4), the maximum and minimum thresholds in temperature (Bio5 and Bio6) and precipitation of wettest and driest quarters (Bio13 and Bio14) were selected.

Niche overlap analyses

The phylogenetic niche conservatism in Arvicolinae subfamily was assessed through the measurement of niche overlap between sister species. For that, the realized niches were compared to quantify the degree of conservation along the phylogeny.

All analyses were performed with the environmental space approach proposed by Broennimann *et al*. (2012). So, the environmental space of each species was defined with the density of occurrences and the corresponding background (R function *ecospat.grid.clim.dyn*, library ecospat; Broennimann *et al*., 2018). In order to consider only environmental conditions potentially accessible to the species (Barve *et al*., 2011), buffer areas of 200 km were created around the occurrence points. The extension of buffer area was a compromise between the

amplitude that could ensure the movement of this kind of species throughout their life and the area that did not exceed the physical limits of environmental data. The abiotic information was extracted as a randomized sample of the 20% of the total number of pixels inside the buffers (Castellanos-Frías *et al*., 2018).

The Schoener's *D* metric was applied to calculate the niche overlap between sister species, which ranges from 0 (no overlap) to 1 (complete overlap) (see Warren *et al*., 2008). In addition, niche equivalency and similarity tests were performed to determine the statistical relevance of the overlap between niches. The equivalency test evaluates whether the niches are identical, thus the occurrences of two species were pooled and randomly reallocated in different datasets in order to calculate news niche overlap (repeated 100 times) (R function *ecospat.niche.equivalency.test*, library ecospat; Broennimann *et al*., 2018). The approach of equivalency test in the version 3.0 has changed compared to previous versions; thereby the alternative hypothesis considers that the niche overlap is more equivalent than random. Then, *D* value from original dataset was compared with the distribution of *D* values from null models; when the observed value was outside the 95% of null distribution the niche equivalency between niches could be assumed. Regarding the similarity test, it evaluates whether the similarity between niches is owed to close environmental conditions in the surrounding territory. The niches were randomized inside the available conditions in the study area and compared between both species (repeated 100 times) (R function *ecospat.niche.similarity.test*, library ecospat; Broennimann *et al*., 2018), to evaluate whether the niches were more or less similar than expected by chance (observed *D* value outside of the 95% of the null distribution).

Additionally, the tests of the niche dynamics (stability, unfilling and expansion) were measured (R function *ecospat.niche.dyn.index*, library ecospat; Broennimann *et al*., 2018). The decomposition of niche in these indices provides detail information about the shifts occurred in the niches (Guisan *et al*., 2014). All measurements are expressed as the comparison between two niches, thus, niche stability indicates the proportion of both niches overlapping. Niche unfilling is the proportion of niche of species one non-overlapping with the niche of the species two. On the contrary, niche expansion shows the section in the niche of species two non-overlapping with the niche of species one.

Null models of the niche overlap tests from nonsister species were developed so as to explore the variability of values within the Arvicoline phylogeny. Thus, the tips from the phylogeny were randomized and the alternative niche overlaps were calculated between nonsisters species (repeated 100 times). As a result, we got a null distribution of values for the different tests by

each node, with which the observed values (from sister species pairs) were compared to. Finally, the statistical significance was calculated. All analyses were executed in R (R Core Team, 2018).

Phylogenetic and ecological distances

Alternatively, according to Losos *et al*. (2003), we examined whether the phylogenetic distances of species were related to the environmental distances of its niches. So, the environmental space was defined with the bioclimatic variables used in the niche overlap analyses and synthesised in a matrix of euclidean distances between species (R function *vegdist*, library vegan; Oksanen *et al*., 2017). Likewise, the phylogenetic distances between Arvicoline species were summarised in a matrix with the function *cophenetic* (R library ape; Paradis *et al*., 2018) as the branch lengths separating species. Both matrices were compared through the Mantel test (R function *mantel*, library vegan; Oksanen *et al*., 2017) to estimate the degree of correlation between them. The null hypothesis of Mantel test postulates that distances in the response matrix are not correlated to the distances in the model matrix (Mantel, 1967) and, hence a lack of correspondence between the phylogeny and the environmental space which is indicative of non-niche conservatism.

Results

The relation of the species with environmental variables was synthesised in a PCA (principal components analysis) axes (Fig. 1). The environmental space explained in average the 73.3% of the variance. Most of the species were related to the increase in the precipitation in the driest month (bio 14) and to the increase in the temperature of the warmest month (bio 5). Half of the sister pairs were close in the PCA diagram, however in the rest they did not follow similar positions.

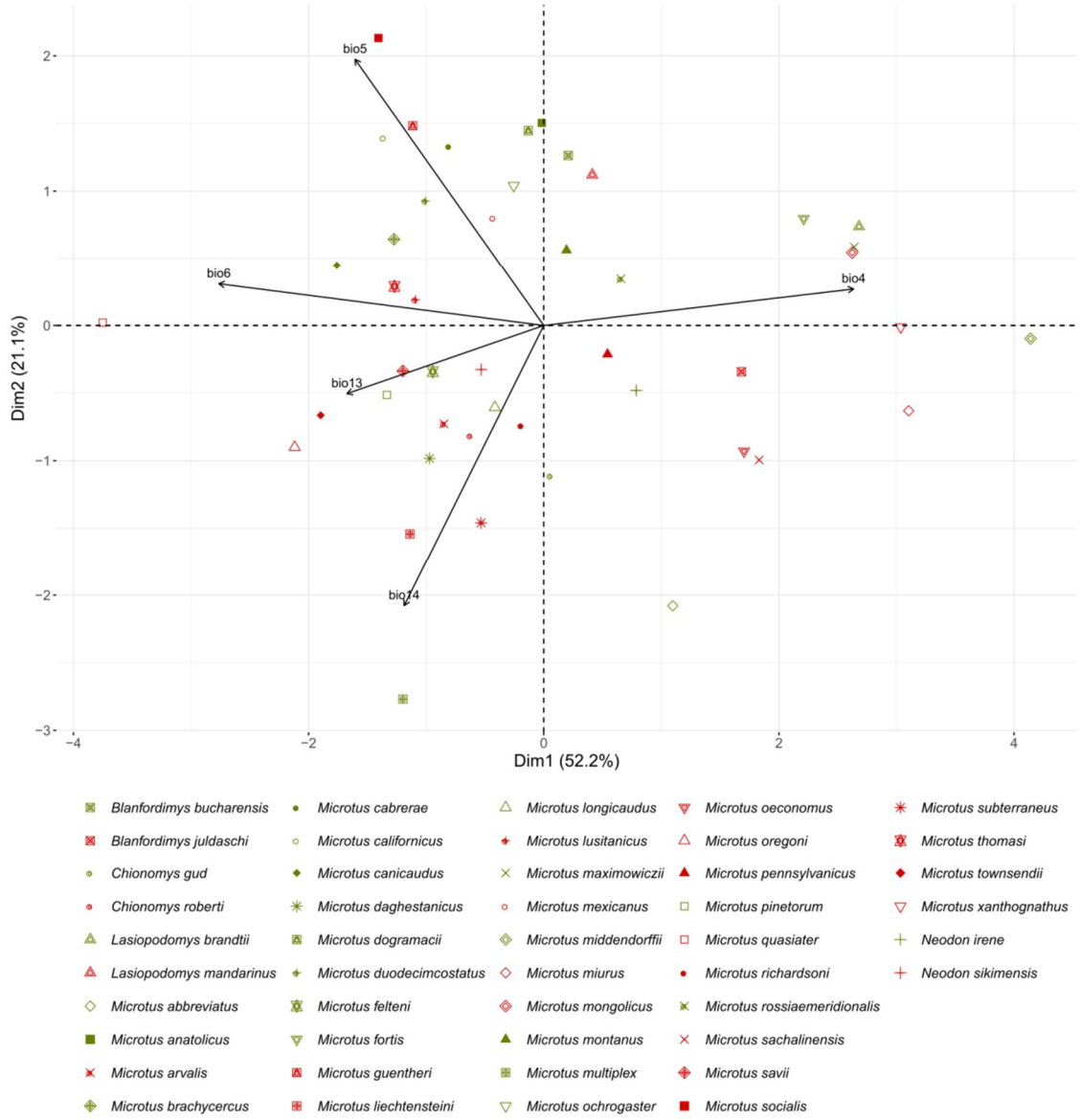

Figure 1. Environmental contribution of each species to the PCA axes. Sister species are represented with the same symbol (one in red and the other in green). Bioclimatic variables: temperature seasonality (bio 4), maximum temperature of warmest month (bio 5), minimum temperature of coldest month (bio 6), precipitation of wettest month (bio 13) and precipitation of driest month (bio 14).

The measurements of niche overlaps throughout the Arvicoline phylogeny became in low values (Table 1), all pairs excepting one revealed lower values than the 50% of overlap. Actually, it is stood out the lowest level of overlap for some sister species pairs, such as *Microtus maximowiczii* - *M. sachalinensis* or *M. middendorffii* - *M. mongolicus* for which the environmental space did not overlap at all or was minimum (Fig. 2). The low overlap of *M.*

maximowiczii - *M. sachalinensis* is due to a methodological limitation, both species are included in the same clade, but they are not originally sister species in the phylogeny, their real sister species had to be omitted because of the lack of distributional data. However, this was the only case of comparisons between non-authentic sister species, the low overlaps of the rest of species should be attributed to real low environmental matches. In this frame, the hypothesis of niche equivalency was rejected for almost all the species pairs, just the pair *M. montanus* – *M. pennsylvanicus* (Table 1, sister species pair: 17) were more equivalent than random. The results of similarity test supported the low level of overlap along the phylogeny. In almost all cases the null hypothesis of niche similarity could not be rejected, therefore the niche overlaps between the niches of sister species could be attributed to regional similarities in the environments of each species. Only four species pairs (Table 1, sister species pairs: 10, 16, 17 and 18) had niches more similar than would be expected by chance, that is, the species of each pair showed a habitat selection of close environmental conditions. Hence, it can be ruled out that the similarity between the niches of these sister species may be caused by analogous conditions to those of the realized niches in the species' backgrounds. The niche dynamics results were in accordance with the values of niche overlap: the sister species with medium to high values of overlap had the highest niche stabilities while, the pairs with the lowest overlap showed the highest niche expansion and/or niche unfilling. The value of niche stability was specially notorious in four sister species pairs: *Neodon irene* - *N. sikimensis*, *M. duodecimcostatus* - *M. lusitanicus*, *M. longicaudus* - *M. oregoni* and *M. montanus* – *M. pennsylvanicus* (Table 1, sister species pairs: 3, 10, 16 and 17, respectively), with stabilities higher than the 90%. In all the pairs the niche overlaps were more similar than random excepting in the pair *N. irene* – *N. sikimensis* which had also high values of niche unfilling, hence its non-similarity of niches (*p-values*: 0.083 and 0.069, for each direction of similarity test).

Table 1. Niche overlap comparisons between sister species of Arvicoline phylogeny. The number of occurrences (N) is collected for each species. The statistical significance of equivalency and similarity tests was conducted with randomization tests. The significant result in niche equivalency means that the overlap between the compared niches is greater than random. In the same vein, the significance for similarity test implies that the overlap between niches is more similar than would be expected from a random distribution of one species created inside its background (Obs.1/Sim.2) or a random distribution of the other species created inside its background (Obs.2/Sim.1).

Sister species	N	Sister species pairs	*D* value	Equivalency test	Similarity test		Niche dynamics		
					Obs.1/ Sim.2	Obs.2/ Sim.1	Expansion	Stability	Unfilling
Chionomys gud – C. roberti	15 - 18	1	0.293	ns	ns	ns	0.560	0.440	0.542
Lasiopodomys brandtii - L. mandarinus	11 - 129	2	0.029	ns	ns	ns	0.469	0.531	0.312
Neodon irene – N. sikimensis	94 - 7	3	0.284	ns	ns	ns	0.019	0.981	0.773
Microtus maximowiczii - M. sachalinensis	123 - 9	4	0.000	ns	ns	ns	0.000	0.000	0.000
M. middendorffii – M. mongolicus	27 - 99	5	0.001	ns	ns	ns	0.929	0.071	0.911
M. fortis – M. oeconomus	110 - 2036	6	0.029	ns	ns	ns	0.878	0.122	0.108
Blanfordimys bucharensis – B. juldaschi	19 - 6	7	0.116	ns	ns	ns	0.747	0.253	0.901
M. daghestanicus – M. subterraneus	10 - 195	8	0.035	ns	ns	ns	0.607	0.393	0.000
M. brachycercus – M. savii	11 - 23	9	0.255	ns	ns	ns	0.564	0.436	0.347
M. duodecimcostatus - M. lusitanicus	2623 - 1095	10	0.505	ns	*	*	0.097	0.903	0.068
M. felteni – M. thomasi	10 - 19	11	0.455	ns	ns	ns	0.424	0.576	0.250
M. multiplex – M. liechtensteini	51 - 23	12	0.142	ns	ns	ns	0.527	0.473	0.559
M. rossiaemeridionalis - M. arvalis	786 - 4508	13	0.181	ns	ns	ns	0.536	0.464	0.360
M. dogramacii – M. guentheri	6 - 8	14	0.004	ns	ns	ns	0.929	0.071	0.061
M. anatolicus – M. socialis	11 - 62	15	0.002	ns	ns	ns	0.968	0.032	0.000
M. longicaudus – M. oregoni	2455 - 485	16	0.328	ns	**	**	0.001	0.999	0.227
M. montanus – M. pennsylvanicus	1741 - 4312	17	0.440	*	**	**	0.070	0.930	0.007
M. canicaudus – M. townsendii	48 - 357	18	0.205	ns	*	*	0.634	0.366	0.002
M. cabrerae – M. richardsoni	316 - 242	19	0.091	ns	ns	ns	0.707	0.293	0.663
M. ochrogaster – M. xanthognathus	1542 - 195	20	0.003	ns	ns	ns	0.918	0.082	0.941
M. californicus – M. mexicanus	1425 - 598	21	0.233	ns	ns	ns	0.272	0.728	0.237
M. pinetorum – M. quasiater	950 - 22	22	0.007	ns	ns	ns	0.747	0.253	0.975
M. abbreviatus – M. miurus	20 - 382	23	0.001	ns	ns	ns	0.999	0.001	0.996

ns (non-significant): $p > 0.05$; *: $p \leq 0.05$; **: $p \leq 0.01$

The results of the low niche overlap in the Arvicoline group were backed by the niche overlap diagrams (Fig. 2). The limited stability extension in the figures (Fig. 2, blue areas) denoted the general tendency to low values of overlap.

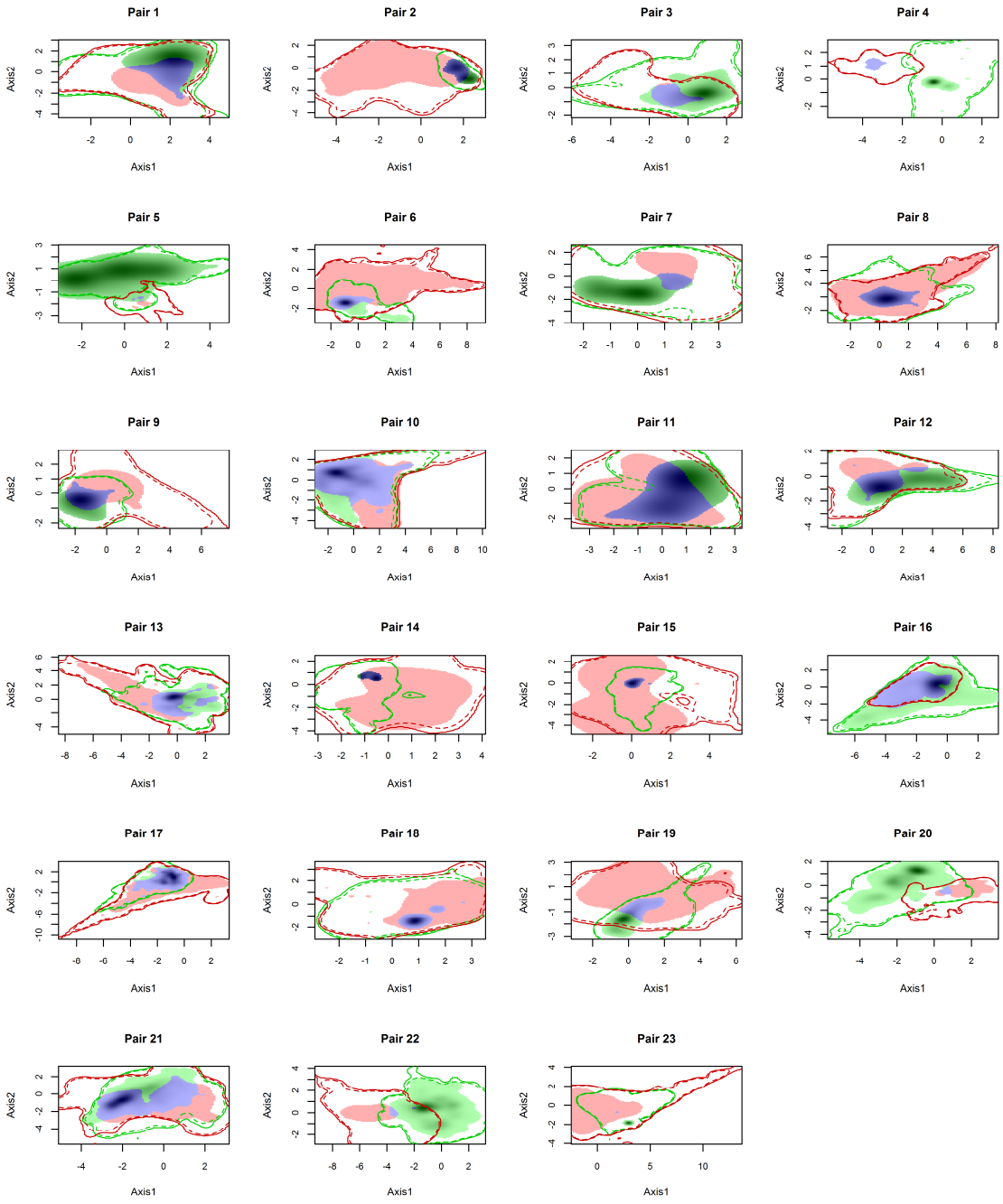

Figure 2. Niche overlaps between the sister species of the Arvicoline phylogeny. The environmental space of each sister species pair is compiled through two PCA axes (red lines for one species and green lines for the other one of the pair). Solid lines represent 100% of available climate, while dashed lines reflect the 50% most frequent available climates. Blue areas indicate stable climatic regions between both species of the pair; the red region denotes the expansion of one species of the pair under its corresponding climate; green pixels indicate climatic zones unfilled by the other species' niche.

The null models revealed niche overlaps not distinguishable from the distribution of niche overlap values of nonsisters species (Fig. 3). Therefore, the results of overlap of a random configuration of species were not significantly different from the observed between sister species of the phylogeny. Only the species pairs: *N. Irene – N. sikimensis*, *M. duodecimcostatus - M. lusitanicus*, *M. felteni – M. thomasi*, *M. longicaudus – M. oregoni* and *M. montanus – M. pennsylvanicus* (p_{pair3} = 0.050, p_{pair10} = 0.010, p_{pair11} = 0.010, p_{pair16} = 0.020 and p_{pair17} = 0.035, respectively) produced niche overlaps statistically different from the D values of the null models. All these species pairs had the higher niche overlap values; hence it was expected to be different from random distributions. Regarding the equivalency and similarity results, only the observed value of the species pair *M. montanus – M. pennsylvanicus* was different in both tests to the values of a random phylogeny ($p_{pair17\ Equivalency}$ = 0.010, $p_{pair17\ Similarity\ Obs.1/\ Sim.2}$ = 0.010, $p_{pair17\ Similarity\ Obs.2/\ Sim.1}$ = 0.010). While in the pair of *M. longicaudus – M. oregoni* only the similarity values were statistically different from the null distribution ($p_{pair16\ Similarity\ Obs.1/\ Sim.2}$ = 0.010, $p_{pair16\ Similarity\ Obs.2/\ Sim.1}$ = 0.010).

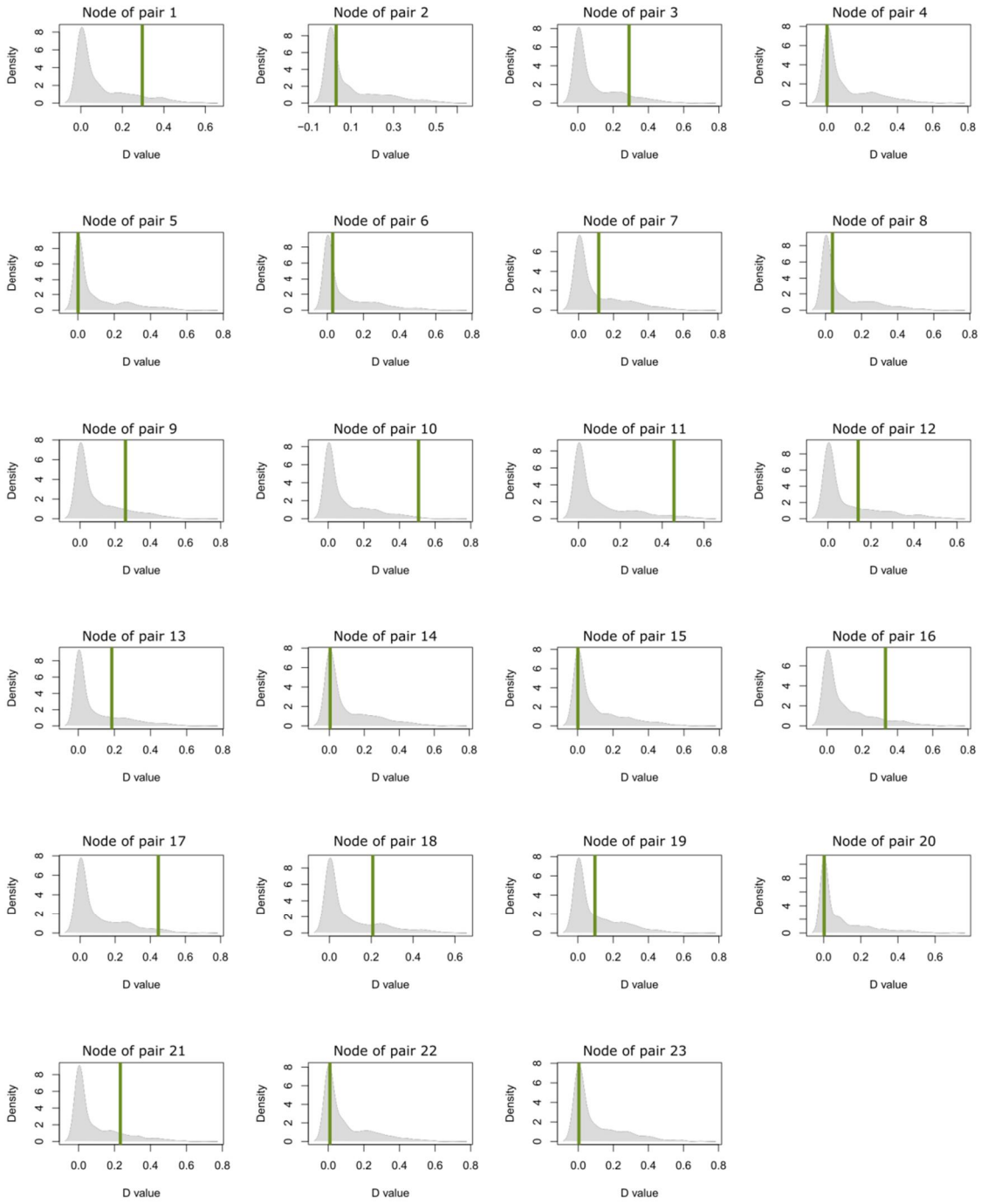

Figure 3. Null distribution of the random niche overlap from nonsisters species. The shadowed area represents the distribution of the *D* values per each node of the randomizations of the phylogeny tips, while the green line is related to the observed *D* value of the original node in the phylogeny.

Additionally, the relation between phylogeny and environmental space was assessed with the Mantel statistic. This methodology revealed that the phylogenetic distances in the Arvicoline

group were not explained by the environmental distances between the species (*Mantel statistic* = -0.042, *p* = 0.704). In accordance with the previous outputs, the Mantel test did not identify statistical significance between the abiotic niches of the species and the phylogeny.

Discussion

The combination of the niche overlap analyses and the distance matrices revealed the lack of niche conservatism throughout the Arvicoline phylogeny. From the niche overlap approach, the comparison between sister species did not show higher niche proximity than may be expected under a random distribution of species. Just in four out of the 23 sister species pairs analysed it was identified a significant overlap. The only species pair whose niches were equivalent and similar was the formed by *M. montanus – M. pennsylvanicus*, that is, both species had identical environmental niches which could not be explained by similar conditions in the backgrounds of these species. Additionally, the species pairs *M. duodecimcostatus - M. lusitanicus, M. longicaudus – M. oregoni* and *M. canicaudus – M. townsendii* had niches more similar to one another than would be expected by chance. Despite the non-equivalency between these niches, the fact that these sister species were similar indicated that each pair tracked analogue conditions within the available space. Notwithstanding, the niche equivalency test is so conservative that in phylogenetic studies is commonly rejected, hence the niche similarity test is more suitable for testing evolutionary hypotheses such as niche conservatism between sister species (Guisan *et al*., 2017). Furthermore, the niche equivalency is highly sensitive to unbalanced number of occurrences between the species. The methodological fundament of this test is based on creating pseudoreplicate datasets from random partitions of the pooled set of occurrences (both species compared), so if a species is much more numerous than the other one, it is more likely to get the data from the former and thereby to get niche configurations analogous to the originals. Therefore, most of the unbalanced datasets may lead to unrealistic niche equivalency measurements. These limitations explained the rejection of the niche equivalency in the species pairs with a high overlap and for which the niche similarity was accepted (Table 1, sister species pairs: 10, 16 or 18).

Likewise, the methodology based on the distance matrices reinforced the results of non-niche conservatism throughout the Arvicoline phylogeny. This approach assesses the phylogeny in a global way, so that the particular correlations between ecological similarity and phylogeny are

not detected. Therefore, the relationships previously identified between some sister species pairs could not be revealed.

Arvicolinae is one of the most diverse lineages of murids, in fact some of their genus, such *Microtus* can present speciation rates up to 60 – 100 times higher to other vertebrates (Triant & DeWoody, 2006). Hence, the non-niche conservatism could be a feasible consequence in a group with high evolutionary rates (Lawson & Weir, 2014). In this vein, our study revealed that most of the species shared the geographical space or were relatively close, however they showed segregation in the environmental space with low niche overlap so that the niche conservatism could not be inferred in these cases. In sharp contrast, the significant niche overlaps of the sister species (*M. duodecimcostatus* - *M. lusitanicus*; *M. longicaudus* – *M. oregoni*; *M. montanus* – *M. pennsylvanicus*; *M. canicaudus* – *M. townsendii*) could be attributed to the fact that these pairs are composed of sympatric species (Douglass, 1976; Verts & Carraway, 1987; Santos *et al*., 2009). Thus, the spatial distribution between these sympatric species can be delimited by subtle differences in soil type, pH (Santos *et al*., 2009) or in vegetation selection (Douglass, 1976). Furthermore, the genetic characteristics of some pair such *M. duodecimcostatus* – *M. lusitanicus* support this pattern, since they have the lowest distance in the *cytochrome-b* between *Microtus* species (Jaarola *et al*., 2004). These four sister species pairs expose the importance of integrating the phylogeny and the environmental space in the study of the niche conservatism (Cooper *et al*., 2011). An analysis of a random species pair living in close proximity should provide a significant overlap, but this overlap may be as a consequence of a convergent adaptation to the common environmental conditions. However, the inclusion of the phylogenetic relationships would clarify the underlying mechanism; so, the fact that the analysis considers sister species is indicative of a conservation of the ancestral niche conditions.

The study of Lv *et al*. (2016) at inclusive phylogenetic scale on the arvicoline rodents showed niche conservatism along the precipitation gradients but not along the temperature variables. This investigation was focused on the reconstruction of ancestral traits of the phylogeny and the calculation of the phylogenetic signal to visualize the niche evolution (Lv *et al*., 2016). Depending on the breadth and the depth of study of the phylogeny (species-, genus-, family- or order-level), the conclusions of niche conservatism or shift can be altered, hence the methods must be coupled to the question to answer (Peixoto *et al*., 2017). In this vein, the phylogenetic scale applied by Lv *et al*. (2016) and in our research did not match, so both results reflected a different aspect of the arvicoline niche. Peixoto *et al*. (2017) identified this lack of analogue when used phylogenetic approaches at the order and family level on bats and geographical approaches at

the species level. The different scales allowed them to find that PNC on bats was supported at the order and family levels but not at the species level. Hence, the evaluation of PNC of arvicoline at broad phylogenetic scale of Lv *et al*. (2016) was completed with ours assess at narrow phylogenetic scale. Therefore, the conclusions from studies of this type must be subject to the scale with which they have been obtained.

The PNC has been studied in other taxonomic groups with diversity of results. In mammals, Olalla-Tárraga *et al*. (2016) analysed the dietary traits and identified phylogenetic signal but not coherence with the Brownian motion (BM) in all dietary categories, so that they could not infer niche conservatism. In Petroicidae family birds it has revealed lack of phylogenetic niche conservatism (García-Navas *et al*., 2018), the same as in a larger study on European birds (Pearman *et al*., 2014). On a Mexican sister taxa (butterflies, birds and mammals) Warren *et al*. (2008) identified non-significant environmental niche conservatism, since the sister species tended to have similar niches but not identical. On the contrary, the phrynosomatid lizards exhibited niche conservatism in precipitation variables with significant signal across the phylogeny (Wiens *et al*., 2013), however other niche variables were not considered. Therefore, the literature that covers the study of the PNC evidences its lack of ubiquitous (Olalla-Tárraga *et al*., 2016).

Notwithstanding, the conclusions about PNC can be affected by the conditions of the research. The choice of the environmental variables used to delimit the species' niche can provide variability on the assumptions of shift or conservatism (Warren *et al*., 2008; Cooper *et al*., 2010). Some environmental variables may be more phylogenetically constrained than others, hence the sensitivity of results may be subjected to the type of variables used. Furthermore, when analysing global sets of species certain variables may not define properly some species' niches. The phylogeny employed can also be a source of uncertainty in the PNC study. The phylogeny is a hypothesis which clarifies statistically the inter-species relationship, although these are not always a true reflection of reality. Identifying the relationship or the position of some species in the phylogeny can be an arduous task, and sometimes the resulting positions have low stability or even become erratic placement in the gene trees (Martínková & Moravec, 2012). This could be the case of some species of our dataset for which the closest relatives are not easily identifiable, causing a lack of resolution of some relationships, such as *M. cabrerae* (Martínková & Moravec, 2012). So, phylogenies with relationships poorly resolved can lead to clusters of erroneous sister species. Additionally, the lack of genetic and distribution studies in some species has generated a data deficit to phylogenetically analyse these species or evaluate their

realized niche (*e.g. M. bavaricus* or *M. transcaspicus*, which were removed from our analyses due to lack of data). As already mentioned, the phylogenetic scale of the study can also condition the conclusions on PNC. The literature collects variations in the conclusions about niche conservatism depending on the approach of the study at deeper or superficial evolutionary scales (Stigall, 2014; Peixoto *et al*., 2017). Therefore, the consideration of the PNC should be circumscribed to the research framework, so that the PNC study should consider the niche conservatism as a continuum (Warren *et al*., 2008) in which the conclusions may be modulated by the study conditions.

In conclusion, the environmental approach on Arvicoline phylogeny has revealed a global pattern of non-niche conservatism. Despite this general trend, the niches of some sister species pairs were not identical, but they were more similar than non-sisters from the same phylogeny. According to Losos *et al*. (2003) and given the short time of divergence of the subfamily one might expect sister species to be ecologically similar, however our results indicated otherwise. The high rate of speciation in the group (Triant & DeWoody, 2006) combined with other factors such as the isolation of some species during the glacial periods (Bilton *et al*., 1998) would explain the lack of conservatism in the phylogeny but the similarity of some taxa. So that, most of the rodents of the subfamily Arvicolinae may have suffered niche shifts, moving away from the conditions of the ancestral niche. Hence, changes in the biotic relationships, niche truncations, lack of equilibrium between the species and the climate or some degree of adaptation to their novel conditions may explain this general trend of non-niche conservatism, nevertheless this ability cannot be assumed for the whole group.

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Discusión general

El análisis de la conservación de nicho es un área relativamente reciente que comienza a desarrollarse a partir de los trabajos de Brown and Pavlovic (1992); Holt and Gaines (1992); Peterson et al. (1999), desde ahí, múltiples investigaciones han tratado de esclarecer este tema (ej.: Wiens & Graham, 2005; Peterson, 2011). Sin embargo, a pesar de la existencia de una amplia literatura que indaga en ello, no se han llegado a identificar los patrones generales de conservación o evolución para los diferentes grupos taxonómicos. De hecho, las revisiones desarrolladas hasta el momento sobre la conservación del nicho concluyen la existencia de evidencias mixtas apoyando tanto la conservación como la evolución del nicho (Wiens & Graham, 2005; Losos, 2008; Pearman *et al*., 2008; Warren *et al*., 2008; Cooper *et al*., 2010; Wiens *et al*., 2010; Peterson, 2011). Peterson (2011) subraya la escala temporal como el principal factor que condiciona los resultados de los estudios de conservación de nicho, así investigaciones con un enfoque temporal a corto plazo tienden a conclusiones de conservación, mientras que evaluaciones basadas en amplias escalas muestran una tendencia a la evolución del nicho. De igual modo, en las aproximaciones filogenéticas el grado de profundidad del estudio sobre la taxonomía (estudios a nivel de especie frente a género, familia u orden) ha sido identificado como un factor que puede conducir a resultados dispares en cuanto a la conservación o no del nicho (Cooper *et al*., 2010). Por tanto, algunos trabajos han encontrado que en evaluaciones a nivel de especie no se tiende a identificar conservación de nicho filogenético, sin embargo sí que se corrobora sobre escalas filogenéticas más profundas de género, familia u orden (Brame & Stigall, 2014; Peixoto *et al*., 2017). No se puede obviar la propia naturaleza del nicho, entendido como un espacio multidimensional, en el que los distintos rasgos pueden haber sufrido diferente grado de evolución o incluso mantenerse conservados (Wiens *et al*., 2010), de ahí que la selección de los rasgos también podría afectar a las conclusiones sobre la conservación de nicho.

En suma, el análisis de la conservación de nicho se encuentra afectado por un amplio abanico de condicionantes, por lo que su habitual evaluación a través de enfoques exclusivamente filogenéticos puede resultar insuficiente (Wiens *et al*., 2010). Los trabajos abordados con esta Tesis exploran la conservación del nicho desde diversas aproximaciones, cubriendo tanto escalas temporales a corto plazo (de individuo o población) como a amplias escalas tanto filogenéticas

como temporales (a lo largo de su historia evolutiva). Gracias a la incorporación de la ecofisiología, las evaluaciones multitemporales (estudio longitudinal) y los análisis a lo largo de la filogenia, en esta Tesis disponemos de una evidencia acumulada con la que responder a la cuestión de la conservación del nicho en una especie de roedor endémico de la península Ibérica, *M. cabrerae*.

El auge en los trabajos ecofisiológicos persigue conocer la respuesta fisiológica de las especies a cambios ambientales y, de este modo extrapolar sus capacidades de respuesta ante cambios climáticos (ej.: García-Muñoz & Carretero, 2013; Sassi & Novillo, 2015). Los estudios ecofisiológicos realizados hasta el momento sobre *M. cabrerae* se han ocupado de describir los parámetros energéticos en el territorio portugués (Mathias *et al*., 2003; Santos *et al*., 2004), sin considerar la variabilidad intraespecífica asociada a los pisos bioclimáticos de distribución de la especie (supra- y mesomediterráneo). A través de los trabajos desarrollados en esta Tesis se subsana dicho déficit (Capítulo 1 y 2). A pesar de que el Capítulo 1 no contribuye a dilucidar la conservación de nicho en la especie, resulta esencial para determinar el comportamiento metabólico de los topillos de Cabrera en la región estudiada y, con ello, fundamentar los subsiguientes experimentos de jardín común. Dado que el calentamiento global es una de las principales amenazas que se cierne sobre la especie, el estudio se ejecutó en el periodo estival a fin de identificar sus estrategias para afrontar condiciones adversas. De este capítulo 1 se desprende que las tasas metabólicas, además de tener un importante componente de base filogenética, se encuentran acopladas a las condiciones ambientales. Así, las condiciones de aridez del verano en la región mediterránea imponen restricciones en el metabolismo basal, fijando niveles más bajos que los identificados en otras especies de microtinos. Por tanto, la estrategia de las poblaciones en las condiciones más cálidas se centra en retrasar el inicio de la zona termoneutra (TNZ) e incrementar la conductividad térmica para disipar el exceso de calor. Adicionalmente, resulta notoria la extensión del rango de la TNZ a lo largo de altas temperaturas, superior a lo detectado en otros arvicolinos (Aalto *et al*., 1993; McDevitt & Speakman, 1994; Chen *et al*., 2012). Las temperaturas superiores a los 24.85º y 26.85º C (límite inferior de TNZ en supra- y mesomediterráneo, respectivamente) son habituales en las poblaciones de *M. cabrerae* durante la estación cálida, de modo que le resulta ventajoso que a partir de esas altas temperaturas el gasto metabólico sea el mínimo. Con este mecanismo la especie minimiza los requerimientos de ingesta de alimento, en un periodo en el que el alimento escasea dentro de los parches de hábitats (Pita *et al*., 2017).

Las experiencias del jardín común (Capítulo 2) señalan que el mecanismo subyacente en el acople fisiológico entre las poblaciones de *M. cabrerae* y las condiciones de los pisos bioclimáticos podría no ser lábil, sino encontrarse fijado genéticamente. De ahí que descartemos que las diferencias intraespecíficas sean producto de la plasticidad fenotípica. No obstante, la falta de éxito en el experimento de cría en cautividad para obtener una generación filial, no nos permite descartar otros mecanismos de ajuste fisiológico no genéticos irreversibles. La reproducción y crianza de animales salvajes bajo condiciones de cautividad es un proceso complejo y en pocas ocasiones se alcanzan los objetivos con éxito. De hecho, hay pocos estudios que hayan logrado obtener generaciones filiares con las que distinguir las diferencias fisiológicas de origen no genético irreversible y las fijadas genéticamente (Spicer & Gaston, 1999). Por ejemplo, Tracy and Walsberg (2001) capturaron hembras preñadas de *Dipodomys merriami* y sometieron a las camadas a condiciones controladas, para determinar que el origen en las diferencias fisiológicas estaba controlado, principalmente, por el mecanismo genético y con una pequeña contribución de la plasticidad fenotípica. Sobre aves, Broggi *et al*. (2005) transfirieron huevos de distintas poblaciones a unas condiciones de cría controladas para identificar que las diferencias metabólicas tenían una base genética. En peces Jensen *et al*. (2008), a través de un experimento de jardín común con cría en cautividad, evidenciaron que las diferencias genéticas entre poblaciones eran la causa de la adaptación local a sus respectivos ambientes. Por tanto, la dificultad aparejada a la reproducción y cría en cautividad de especies silvestres, limita la realización de estudios con los que excluir mecanismos genéticos de los no genéticos a especies de fácil manejo y, con ello, extrapolar conclusiones sesgadas a especies con características muy concretas.

La proliferación de estudios intraespecíficos sobre una diversidad de taxones ha permitido aportar evidencias sobre el potencial adaptativo presente en las tasas fisiológicas (BMR o RMR). En ellas se identifica una componente ambiental y otra genética que les permite a las especies tolerar la variabilidad ambiental de sus nichos (Spicer & Gaston, 1999). Sin embargo, las presiones selectivas impuestas por el medio pueden no desencadenar diferenciación fisiológica de base genética o que las tasas metabólicas en ciertos grupos tengan una fuerte inercia filogenética (Cruz-Neto *et al*., 2003), de modo que en esos taxones la incapacidad de respuesta adaptativa en sus rasgos fisiológicos frente al cambio climático puede comprometer su supervivencia. En cambio, según revela nuestro trabajo (Capítulo 2) las tasas metabólicas de *M. cabrerae* podrían adaptarse a cambios graduales de su nicho fundamental a causa del cambio climático. No obstante, la especie es un especialista del hábitat que encuentra las mayores restricciones bajo las condiciones de aridez que impone el verano de la región mediterránea. En

este periodo la humedad freática disminuye y se reducen los parches de vegetación que proporcionan guarida y alimento a la especie. De forma análoga a las restricciones del verano mediterráneo, un incremento de la aridez como el previsto con el cambio climático, además de aproximar las condiciones ambientales al límite fisiológico soportado por este taxón, muy probablemente contribuirá a la pérdida y reducción de los hábitats disponibles, comprometiendo así la estabilidad de la especie.

Estas mismas conclusiones son las que se derivan de la transferencia temporal de paleo-modelos y la evaluación del espacio ambiental ocupado por la especie a lo largo de su historia (Capítulo 3). El empleo de un doble enfoque metodológico nos ha permitido reforzar los resultados de este capítulo. Del análisis geográfico se extrae un fracaso en la transferencia temporal entre los paleo-nichos y el nicho actual. Al igual que reseña la literatura, coincidimos en que en la ejecución de los modelos de distribución de especies (SDM) intervienen numerosos factores que condicionan sus resultados (ej., el sobreajuste de los modelos). De ahí que un análisis complementario basado en el espacio ambiental permitió esclarecer las causas ambientales que motivaron el cambio en el nicho realizado de la especie.

Las evaluaciones de equivalencia a lo largo de la historia de esta especie revelan que los nichos ocupados en los distintos escenarios no son equivalentes. En cambio los análisis de similitud mostraron que, de forma parcial durante el Último Máximo Glacial (LGM) y totalmente en el Último Interglacial (LIP) los nichos realizados fueron similares. En este último periodo la especie quedó relegada a condiciones ambientales más restringidas que las de la actualidad.

El rechazo de la equivalencia en las comparaciones entre nichos es una tónica general en los trabajos que evalúan el solapamiento de nicho, por ejemplo, según justifican Warren *et al*. (2008) en su investigación: “los nichos de las especies hermanas son raramente idénticos, pero tienden a ser más similares” (p. 2879). De acuerdo con Guisan *et al*. (2017), el test de equivalencia es tan restrictivo que habitualmente es rechazado, por lo que los propios autores recomiendan emplear el test de similitud para evaluaciones en los nichos con un enfoque filogenético o multitemporal. El método de cálculo del test de equivalencia le convierte en un índice muy sensible a desbalances en el número de presencias de los nichos a comparar. Al respecto, Peterson (2011) considera que este test incurre en errores estadísticos de Tipo I, puesto que suele rechazar la hipótesis nula cuando no es lo que en la realidad ocurre. Por tanto, tras todos los análisis desarrollados en base a esta metodología fundamentada en los trabajos de Warren *et al*. (2008) y Broennimann *et al*. (2012) (Capítulo 3 y 4), creemos que los resultados del test de equivalencia no son fiables y, a pesar de que podría proporcionar una información de

gran interés, el diseño matemático que le sostiene le convierte en un test que aporta ambigüedad, de modo que desaconsejamos su uso.

Independientemente de las cuestiones puramente metodológicas, el análisis de la respuesta de los taxones frente de los cambios climáticos acontecidos a lo largo de su historia evolutiva puede proporcionar una valiosa información para determinar sus capacidades y limitaciones y, así, esclarecer sus estrategias de supervivencia. Durante el periodo de glaciación y el Holoceno Medio la especie presenta nichos realizados con condiciones ambientales ligeramente diferentes a las que es capaz de soportar en la actualidad. De ahí que se deduzca que podría desarrollar cierta capacidad de adaptación genética o presentar plasticidad fenotípica que le permitiese expandir sus umbrales de tolerancia fisiológica a condiciones contrastadas. Sin embargo, esta capacidad quedaría limitada durante un escenario de calentamiento como el que representa el Último Interglacial, en el que la especie redujo su espacio ambiental ocupado con respecto al actual. Las condiciones climáticas acontecidas durante el Último Interglacial provocaron una tendencia similar en especies adaptadas al frío como el mamut lanudo (*Mammuthus primigenius*) que sufrió una fuerte reducción en el área climáticamente idónea (Nogués-Bravo *et al*., 2008), la contracción del nicho en *Sigmodon* spp. (Hadly *et al*., 2009) o el desplazamiento hacia latitudes septentrionales de especies de megafauna americana como el mastodonte (*Mammut americanum*), el perezoso gigante (*Megalonyx jeffersonii*) o el castor gigante (*Castoroides ohioensis*) (Zazula *et al*., 2014). A pesar de que las condiciones de calentamiento del Último Interglacial provocaron la contracción o desplazamiento del nicho de algunas especies, este efecto negativo no derivó en un periodo de relevantes extinciones. Al tratar de extrapolar los efectos del LIP al escenario de calentamiento global actual, es importante considerar que el marco cuenta con un factor clave que no estaba presente durante el LIP: el ser humano. El factor antrópico genera una degradación y pérdida de hábitats junto con la reducción directa de ciertas poblaciones de especies, que sumado a los cambios del clima podría desencadenar contracciones de nicho mucho más severas que las acontecidas durante el LIP e incluso llegando a provocar extinciones. Según las estimas de la Plataforma Intergubernamental de Ciencia y Política sobre Biodiversidad y Servicios Ecosistémicos (IPBES, por sus siglas en inglés) la actividad humana podría conducir a la extinción de hasta un millón de especies de plantas y animales en un corto plazo (Tollefson, 2019). Sin embargo, hay que tener en cuenta que estas estimas, basadas en la información publicada, puede ser excesivamente catastrofista dado que muchos de esos trabajos se sustentan en las premisas de conservación del nicho. Este hecho realza más la importancia de obtener estimas más realistas del riesgo de extinción que tengan en cuenta escenarios alternativos de respuesta de las especies al cambio ambiental.

No obstante, no se pueden descartar otros procesos que podrían explicar el cambio del nicho a lo largo de la historia de *M. cabrerae*, como el truncamiento del nicho o la falta de equilibrio de la especie con el clima. La transferencia espacial de los SDMs actuales muestra que el sureste francés presenta las condiciones de idoneidad climática para la especie, pero no es un territorio incluido en su nicho realizado, probablemente a causa de interacciones bióticas (Capítulo 3). Por ello la falta de equilibrio de la especie con el clima puede estar afectando a su nicho realizado actual y, por ende, a la distribución de la especie en los diferentes paleo-escenarios climáticos. Esta falta de equilibrio es la causa a la que recurren Varela *et al*. (2009) para explicar el fracaso en la transferencia temporal y espacial de sus SDMs sobre la hiena moteada (*Crocuta crocuta*), ya que a partir de su distribución actual no consiguen predecir la paleo-distribución en la región euroasiática. Así, el modelado de especies cuyos nichos no se encuentran en equilibrio con el clima puede conducir a predicciones de áreas de idoneidad más acotadas que las realmente toleradas por la especie, por ello, estos casos deben ser tratados con extrema cautela. En el mismo sentido, el truncamiento del nicho obvia la adaptación de las especies a condiciones que no se derivan de su nicho realizado actual. De ahí que algunos autores recomienden emplear el registro histórico completo de la distribución de la especie de estudio para determinar todo el espectro climático tolerado y, así, sortear los problemas de modelado derivados del truncamiento del nicho (Varela *et al*., 2010; Maiorano *et al*., 2013). El problema de este enfoque metodológico reside en su asunción de la invariabilidad de los rasgos fisiológicos a lo largo de la historia evolutiva de la especie. Al aplicar esta fórmula de modelado se estaría considerando la ausencia de alteraciones genéticas en la tolerancia fisiológica de la especie durante una amplia ventana temporal (ej., de 130,000 años), a pesar de los cambios climáticos tan contrastados que ha incluido.

En línea con el resto de capítulos de esta Tesis, la metodología basada en las relaciones filogenéticas (Capítulo 4) también respalda los resultados de ausencia de conservación de nicho en la filogenia de los arvicolinos. En el 83% de las especies hermanas estudiadas revelan unos niveles de solapamiento de nicho muy bajos, que implica la ausencia de conservación de nicho filogenético. Sólo cuatro pares de especies hermanas muestran un nivel de solapamiento significativo, indicativo de que en ellas se ha producido una selección de espacios ambientales similares a las condiciones del nicho ancestral.

En lo que respecta a *M. cabrerae*, el análisis del solapamiento en la filogenia muestra un nivel de solapamiento muy bajo de ésta con su especie hermana, *M. richardsoni*, una especie distribuida a lo largo del noroeste de Norte América. De acuerdo con la literatura que aborda su filogenia,

M. cabrerae podría haberse escindido del género *Microtus* en las etapas tempranas de la radiación del grupo, de ahí que resulte complejo establecer sus relaciones de parentesco (Martínková & Moravec, 2012). Por tanto, los diferentes estudios que han tratado de reconstruir la filogenia del grupo han emparentado al topillo de Cabrera con las especies de *Microtus* del Neártico (Jaarola *et al*., 2004; Martínková & Moravec, 2012). Martínková and Moravec (2012) achacan este agrupamiento a una combinación de artefactos estadísticos en la construcción de la filogenia y los procesos estocásticos sobre el ADN acontecidos en la evolución de la especie. Alternativamente, un reciente estudio filogenético centrado en los microtinos (Barbosa *et al*., 2018) rechaza este vínculo de *M. cabrerae* con las especies Neárticas y lo incluye entre los clados Paleárticos. Según este trabajo, las peculiaridades de *M. cabrerae* se achacarían a la antigüedad del linaje, que habría sufrido un largo tiempo de divergencia respecto a los otros subgéneros *Microtus*, o debido a un proceso de evolución acelerada en respuesta a fuertes presiones selectivas y múltiples cuellos de botella, como consecuencia de su especialización ecológica. . Por ende, a pesar de las discrepancias filogenéticas sobre el topillo de Cabrera, parece que las condiciones de frío y hielo imperantes durante la última glaciación impusieron un aislamiento de las especies mediterráneas en áreas como la península Ibérica, que podrían haber propiciado que especies como *M. cabrerae* acumularan nuevas mutaciones y, con ello, cambios adaptativos, que habrían contribuido a sus peculiaridades filogenéticas (Bilton *et al*., 1998).

El análisis global de los experimentos e investigaciones desarrolladas en esta Tesis nos conduce a concluir que la conservación del nicho no es una asunción que se cumpla en *M. cabrerae*. A pesar de no haber ejecutado ningún estudio genético, otras investigaciones que si los han desarrollado, han puesto de manifiesto la existencia de ciertas peculiaridades genéticas, indicativas de un proceso de especiación en los topillos de Cabrera que le hubiera alejado de las condiciones del nicho ancestral. Por tanto, la asunción de la conservación de nicho debe ser una cuestión a reconsiderar en función del taxón o el marco de estudio, pero bajo ningún concepto debería seguir siendo tratado como una cualidad inherente a las especies. En este sentido, los futuros trabajos que estudien la conservación de nicho deben seleccionar especies o grupos de especies de contrastadas estrategias vitales (ej. especialistas, generalistas, con ciclos biológicos lentos o rápidos) a fin de identificar posibles patrones. Así mismo, estos estudios deben abordar un amplio enfoque metodológico que permita cubrir las deficiencias que ofrecen algunas técnicas usadas individualmente (ej. ciertas limitaciones de los SDMs). A pesar de las dificultades que pueden plantear los estudios fisiológicos, sus conclusiones pueden ser muy esclarecedoras, por lo que siempre deberían estar contemplados en este tipo de trabajos. No obstante, una buena previsión de las posibles limitaciones de estos estudios fisiológicos puede subsanar sus

deficiencias, así un manejo previo del taxón aporta información de gran interés en la selección de los experimentos más apropiados (estudios de jardín común y/o transplantes recíprocos). De igual modo, el análisis del espacio ambiental ocupado por los taxones a lo largo de su historia evolutiva puede dilucidar los límites reales de las especies para hacer frente a los cambios climáticos. Sin embargo, se deben extremar las precauciones a la hora de aplicar tanto los análisis de los espacios ambientales como las transferencias temporales de los SDMs en situaciones de registros fósiles incompletos, falta de equilibrio con el clima o truncamientos de nichos, pues pueden conducir a resultados sesgados. Finalmente, las evaluaciones filogenéticas permiten contextualizar los taxones con sus grupos, de ahí que deban incluirse a través de diferentes escalas taxonómicas para obtener resultados concluyentes sobre la conservación del nicho.

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Conclusiones generales

- Las mayores restricciones sobre la fisiología de la especie vienen determinadas por las condiciones de aridez impuestas por el verano de la región mediterránea, de modo que la especie extiende la zona de termoneutralidad hacia las temperaturas más cálidas, con lo que minimiza el gasto metabólico, e incrementa la conductividad térmica para disipar el exceso de calor.

- Existe un acople entre los rasgos fisiológicos y el piso bioclimático de origen de los topillos de Cabrera. Esta diferenciación fisiológica intraespecífica se achaca a modificaciones genéticas como las generadas por la adaptación genética local, aunque no pueden descartarse ajustes fisiológicos no genéticos irreversibles.

- El fracaso en la transferencia temporal de los modelos de distribución de especies entre el nicho actual y los paleo-nichos es el primer indicio de cambio en el nicho de la especie. El enfoque del espacio ambiental ocupado por la especie a lo largo de su historia evolutiva respalda estas conclusiones de no conservación del nicho.

- La comparación con los paleo-nichos del Holoceno Medio y del Último Máximo Glacial revelan que el nicho de la especie cambió hacia condiciones ambientales diferentes a consecuencia de la adaptación o plasticidad fenotípica frente a los cambios climáticos. En cambio, el paleo-nicho durante el Último Interglacial presenta un nicho más restringido pero de similitud significativa con el nicho actual de la especie. Esta contracción en el paleo-nicho indica que bajo un escenario de calentamiento, como el representado en ese periodo, los parámetros fisiológicos de la especie podrían encontrarse próximos a su límite de tolerancia y la capacidad de adaptación de especie superada.

- El bajo nivel de solapamiento entre los nichos de las especies hermanas de la filogenia de arvicolinos revela una tendencia de no conservación del nicho ancestral. Sólo en 4 pares de especies hermanas los nichos han conservado las condiciones ancestrales y son nichos más similares de lo que lo que se podría esperar por azar.

- Las peculiaridades genéticas de *M. cabrerae* hacen que se le emparente con especies de microtinos de la región Neártica, con las que comparte muy bajos niveles de solapamiento de nicho. De ahí que el análisis filogenético del nicho de la especie también respalde los resultados de no conservación del nicho ancestral.

- El análisis conjunto de los experimentos ecofisiológicos y la evaluación de los espacios ambientales a lo largo de la historia evolutiva de la especie y en su filogenia, nos aporta una evidencia acumulada para descartar la conservación del nicho en *M. cabrerae*. Por todo ello, la asunción de la conservación de nicho no puede ser una cualidad inherente a las especies.